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MILITARY BLOOD BANKING 1941-1973

**LESSONS LEARNED APPLICABLE TO CIVIL DISASTERS AND
OTHER CONSIDERATIONS. (TARAWA: '6,000 PINTS OF
PLASMA WENT ASHORE WITH THE INVADING TROOPS AND
4,000 PINTS CAME BACK IN THE VEINS OF WOUNDED
MARINES')**

ARMY MEDICAL RESEARCH LABORATORY

1973

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Military Blood Banking	Universal Donor							
Blood Research	History							
Blood Bank Training	Mass Casualty							
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Military blood bank experiences are reviewed, especially the highlights encountered in the period 1941-1971 during World War II, Korean and Vietnam campaigns. The Vietnam statistics correlate well with COL William H. Crosby's observations and recommendations in Korea concerning group O universal donor blood versus group specific, crossmatched blood. There were no reported deaths in Vietnam in over 100,000 group O universal donor transfusions, how- ever, group specific, crossmatched blood given during mass casualty situations (cont)								

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resulted in 49 reported cases of hemolytic transfusion reactions and nine deaths. Mannitol and hemodialysis were effective in salvaging the other cases of hemolytic transfusion reaction. The activation, including staff study and growth of the Blood Transfusion Division and The Blood Bank Center of the US Army Medical Research Laboratory, Fort Knox, Kentucky, is used as an example of planning for large-scale blood banking in the military (operations, training, and research) and, consequently, civil disaster blood bank planning. Large-scale blood bank operations in being at Fort Knox are illustrated and described. The growth and rapid expansion at Fort Knox paralleled the Vietnam campaign in both timing and activity. The contributions of MG Douglas B. Kendrick, BG Robert M. Hardaway, III, and COL William H. Crosby are discussed throughout the monograph.

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MILITARY BLOOD BANKING 1941-1973
LESSONS LEARNED APPLICABLE TO CIVIL DISASTERS
AND OTHER CONSIDERATIONS

(Tarawa: "6,000 Pints of Plasma Went Ashore With the Invading Troops and
4,000 Pints Came Back in the Veins of Wounded Marines.")*

A Monograph

by

Colonel Frank R. Camp, Jr., MSC, USA

Colonel Nicholas F. Conte, MC (M.D.), USA

and

Lieutenant Colonel Jerry R. Brewer, MSC, USA

1973

The Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

*Kendrick, D. B. Blood Program in World War II. Washington, D. C.:
Office of The Surgeon General, Department of the Army, 1964, p. 59.

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Brigadier General Kenneth R. Dirks, MC (M.D.)

Commanding

US ARMY MEDICAL RESEARCH LABORATORY

Colonel Frank R. Camp, Jr., MSC

Commander/Director

1973

Library of Congress Catalog Card No. 78-184862

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Nicholas F. Conte



Jerry R. Brewer

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Lieutenant Colonel Jerry R. Brewer, MSC:

Member, American Association for the Advancement of Science; Member, American Association of Blood Banks; Member, American Chemical Society; Member, International Society of Blood Transfusion.

PREFACE

There were numerous reasons for undertaking a study of military blood banking operations applicable to civil disaster. Not the least of these was the problem existing in emergency rooms of large and small medical centers in the United States. Specifically, this pertains to the requirement for handling five, ten, or 15 casualties resulting from knife, gunshot, or related injuries for which blood is initially indicated. The decision to resuscitate these casualties with blood or expanders is a problem addressed in this monograph. It is hoped that sufficient information has been presented to resolve this problem adequately.

15 June 1973

Fort Knox, Kentucky

Frank R. Camp, Jr.

Nicholas F. Conte

Jerry R. Brewer



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IN MEMORIAM



Dr. Morten Grove-Rasmussen

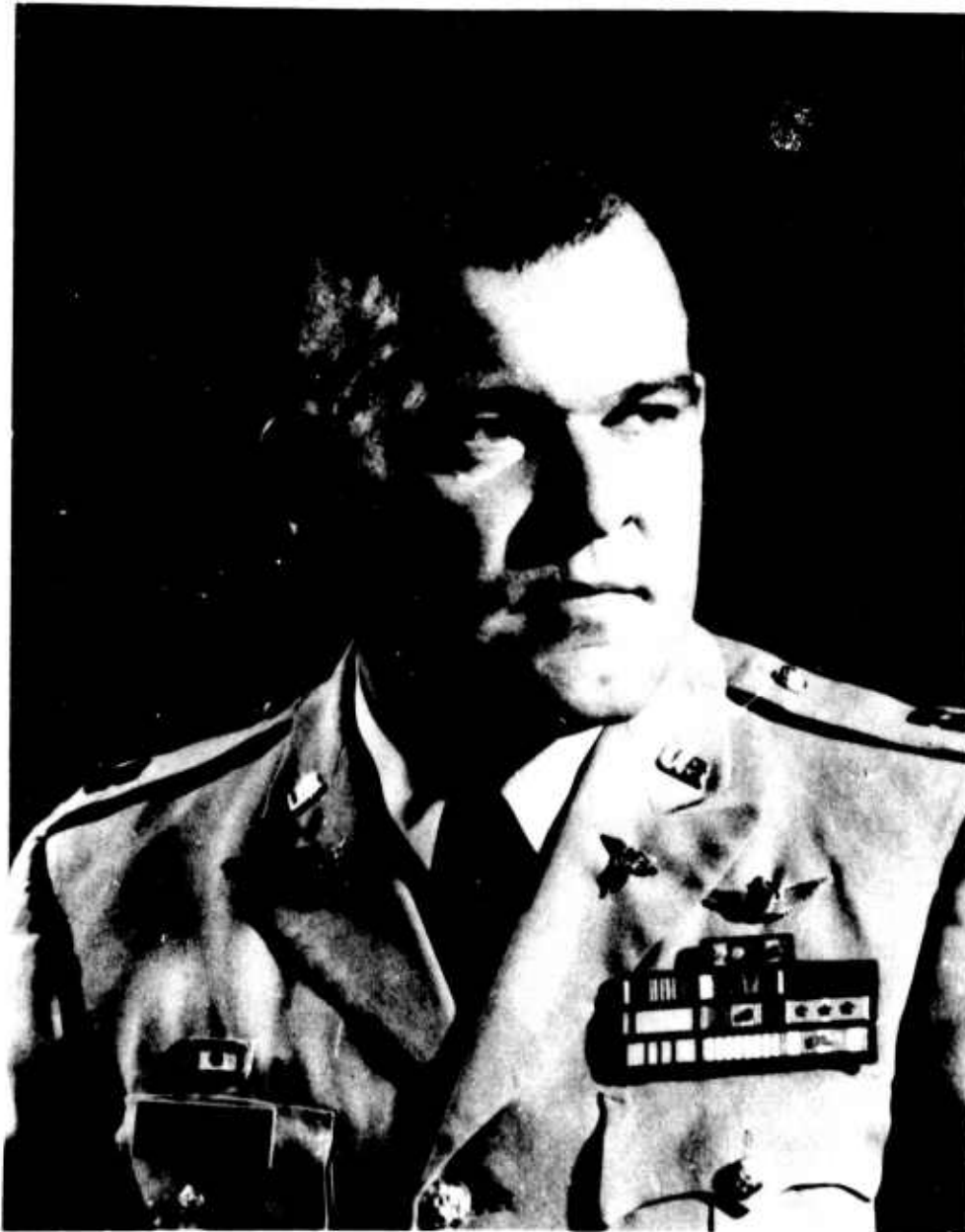
1912-1973

Dr. Grove-Rasmussen contributed much to military blood banking in the areas of blood grouping, blood banking, and blood transfusion.

This includes his early recognition of the problems associated with group O universal donor blood, especially the significance and relationship of isoagglutinins A and B, immune A and B, and hemolysins A and B; his assistance in the translation of Dr. Arne Gammelgaard's thesis on weak subgroups of A blood; and his encouragement for this laboratory to join the Reference Laboratory Program of the American Association of Blood Banks.

Finally, we must also acknowledge the group O red cell antigen combination for low temperature preservation suggested by Dr. Grove-Rasmussen and Dr. Charles Huggins.

IN MEMORIAM



Major J. C. Rothwell, MSC

1932-1969

This efficient and dedicated officer contributed to areas of operations, training, and research, during peace and war, in military medicine and military blood banking.

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IN MEMORIAM



CWO Joseph C. Byrnes

First Administrative Assistant to The Blood Bank Center, 1965.

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IN MEMORIAM



COL Joseph H. Akeroyd, MSC



LTC Edgar B. McCord, MSC



LTC Joseph A. Walkowski, MSC

Scientists - Teachers - Friends

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PRESENT AND PAST OFFICERS ASSIGNED TO THE
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COL James E. McCarty, MC
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*USARV Blood Program Officer.

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Lt Alan H. Runck, MSC, USNR
Ens W. P. Monaghan, MSC, USN
Ltjg Carol Bink, MSC, USNR
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Cdr Joseph G. Gregonis, MC, USN

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(1966 to Present)

ARMED SERVICES WHOLE BLOOD PROCESSING LABORATORY

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Lt Donald R. Levan, MSC, USN

CPT Robert Sarnowski, MC, USAF

Lt William Stith, MSC, USN

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COL Hal G. Etter, BSC, USAF

MAJ Sherwood Hill, BSC, USAF

CPT Anthony G. Cumuze, Jr., BSC, USAF



Captain Lloyd R. Newhouser, USN

Captain Newhouser was in the midst of the action concerning military blood banking in World War II. His contributions to the World War II program distinguished him as a pioneer in blood banking as we know it today.

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COL J. F. Metzger, MC (M.D.)

Colonel Metzger was Commander of the 406th Medical General Laboratory during the heavy support periods of the Vietnam Conflict and contributed to improved blood logistics.

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**ACTIVATION AND GROWTH OF BLOOD PROGRAMS
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121**

*1964 Staff Study
1965 Blood Transfusion Research Division
1965 Blood Group Reference Laboratory
**1965 Quality Control Monitoring (DPSC)
1965 Blood Bank Fellowship Program (3 Fellows) Army
1966 Medical Corps Officer Training Program
1966 Reference and Forensic Testing Laboratory
1966 Blood Transfusion Division
1967 Institutional Membership, AABB
***1967 Approved Institution of Training AABB-ASCP
1969 Blood Coagulation Laboratory
1969 Transfusion Reaction Model
1969 Blood Components Center
1969 Blood Bank Fellowship (4 Fellows) 3 Army, 1 Navy
1970 Histocompatibility (Lymphocyte Typing) Laboratory
1970 Field Testing Laboratory
1970 311-F1 Blood Bank Training for Enlisted Personnel
1971 The Blood Bank Center
1971 Blood Research Division
1971 AABB Reference Laboratory
1971 Blood Bank Fellowship (5 Fellows) 3 Army, 1 Navy, 1 Air Force
1972 HAA Testing Reference Laboratory - US Army
†1972 Blood Bank Fellowship (7 Fellows) 4 Army, 1 Navy, 2 Air Force
1972 Frozen Red Blood Cell Bank
1972 Expansion of Forensic Testing Laboratory Capability
1973 Rare Donor Registry
1973 Affiliated Campus with Bowling Green State University for
Advanced Degrees (MS, BIO. S., Ph. D.)

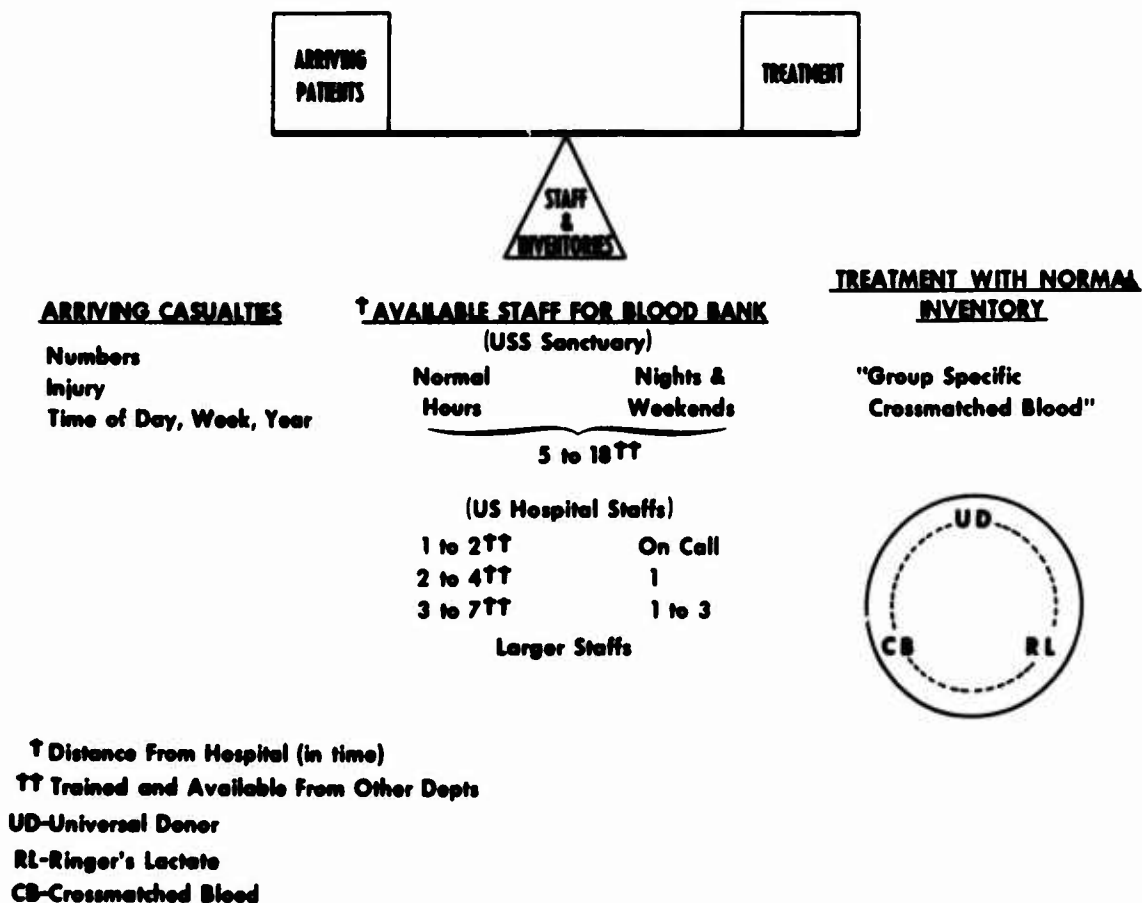
FUTURE GOALS

Blood Bank Traineeship Program for NON Comissioned Officers
Special Studies Laboratory
Reference Laboratory for Military Exigencies & TOE 8-500
Field Testing Service
Hepatitis Registry

*Crosby & Camp
**Defense Personnel Support Center
***American Association of Blood Banks
***American Society of Clinical Pathology
†Program of Accreditation of the AABB and the AMA through the
Board of Schools (ASCP)
‡Specialist Applied Biology (Immunohematology)

FRONTISPIECE A

24 HOUR TREATMENT CENTER FOR MULTIPLE CASUALTIES - A MODEL *

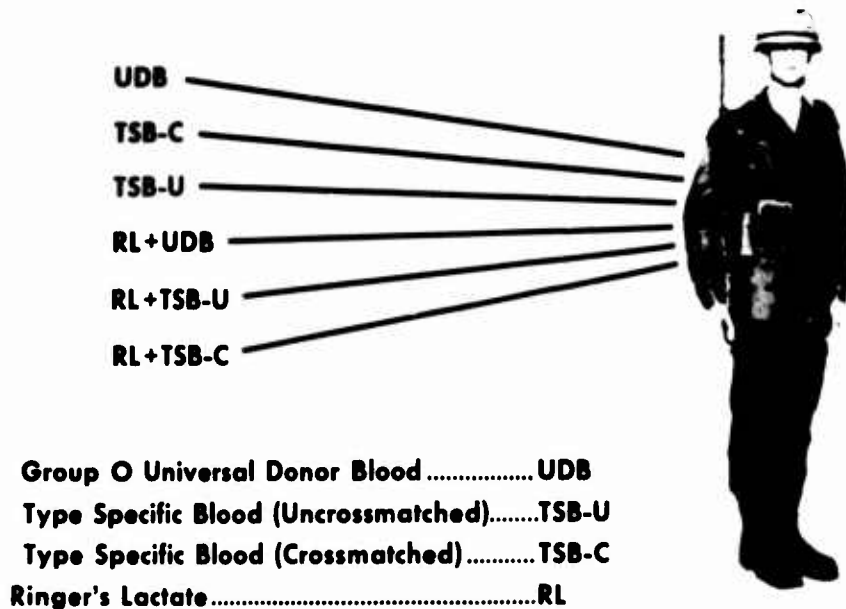


In this frontispiece we attempted to illustrate the range of capability inherent in various treatment facilities. If we consider the staff and inventories triangle to be the fulcrum, the arriving patients as the weight, and the treatment methods as the applied pressure, then it can be shown that variables can make a seesaw situation. At times, the Blood Transfusion Officer must make the decision to shift from business-as-usual to an emergency set of procedures.

*Model: From "Blood Transfusion Aboard a Naval Hospital Ship Receiving Multiple Casualties in the Combat Zone," by W. P. Monaghan, D. R. Levan, and F. R. Camp, Jr. (in manuscript).

FRONTISPIECE B

TIME-RELATED EMERGENCY METHODS FOR MASS CASUALTY SITUATIONS



The sorting team chief should be in a position to know the number of casualties the hospital can accommodate, and this information should be available to the professional staff and blood bank. This variable is influenced by the size of the medical installation. Even with training and experience, these are difficult decisions.

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MONOGRAPH HISTORY

- 1968 Decision made to prepare 3-War blood program monograph.
- 1968 Compilation of Vietnam monthly reports begun.
- 1968-70 World War II and Korean War blood data prepared.
- 1971 (Mar) Vietnam compilation table prepared (331-71).
- 1971 (Sep) Universal donor findings in Vietnam discussed with Dr. Richard E. Rosenfield, who commented, "Actually, you have confirmed what Crosby found in Korea."
- 1971 (Oct) Monograph reviewed by COL Nicholas F. Conte, MC (M.D.).
- 1971 (Nov) Library of Congress Catalog Card Number 78-184862 obtained.
- 1971 (Dec) Monograph reviewed by MG Douglas B. Kendrick, MC (M.D.), USA (Ret).
- 1972 Verification of listing of Vietnam blood program officers begun:
- a. Asa Barnes, M.D. (5 Apr 72).
 - b. COL Kenneth R. Dirks, MC (M.D.) (6 Apr 72).
 - c. US Air Force and US Navy officials.
- 1972 (May) Monograph reviewed by MAJ Jerry R. Brewer, MSC.
- 1972 (Sep) Monograph and universal donor findings discussed with COL William H. Crosby, MC (M.D.), USA (Ret), who provided additional references on shock research.
- 1973 The history of the Fort Knox programs in blood research, operations, and training completed.

DEDICATION

This monograph is dedicated to the staff, present and past, of the Blood Transfusion Research Division, Blood Transfusion Division, Blood Research Division, and The Blood Bank Center; to the Commanders, US Army Medical Research and Development Command and US Army Medical Research Laboratory and their staffs; and to the many military leaders, scientists, teachers, and friends in the United States and abroad who created and sustained the effort in military blood banking at the Fort Knox laboratories.

In particular, it is dedicated to Colonel William H. Crosby, MC (M.D.), USA (Ret), Major General Douglas B. Kendrick, MC (M.D.), USA (Ret), and Brigadier General Robert M. Hardaway, III, MC (M.D.), USA--our mentors.



William H. Crosby



Douglas B. Kendrick



Robert M. Hardaway, III

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BASIC DEFINITIONS

Mass casualty (medical standpoint): An overwhelming number of seriously injured or otherwise incapacitated individuals, occurring within a brief period of time, within a limited area or multiple areas, placed upon locally available medical facilities completely unable to supply conventional medical care for them.

Triage: An evaluation and classification of casualties for purposes of treatment and evacuation. It is the immediate sorting of patients according to type and seriousness of injury, likelihood of survival, and the establishment of priority for treatment and evacuation to assure medical care of the greatest benefit to the largest number.

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SECTION I

HISTORIC BACKGROUND (1-23*)

The period 1914-1917 is interesting in that Lewisohn used blood collected in sodium citrate at Mount Sinai Hospital in New York and published his findings in 1915. Rous and Turner described their solution of sodium citrate and dextrose in 1916; and Major Oswald H. Robertson set up the world's first military blood bank in 1917. He collected 500 ml of group O blood in Rous-Turner solution and stored the blood bottles in a refrigerator. Major Robertson transfused the blood to patients in Casualty Clearing Stations and Advanced Dressing Stations of the British Army. The blood was used up to 14 days.

During World War II, blood research was again intensified, and in 1940, DeGowin *et al* developed a modification of the Rous-Turner solution. This was followed in 1943 by the Denstedt, Alsever's, and Loutit-Mollison solutions. Initially, one-liter glass bottles containing 500 ml of Alsever's solution and 500 ml of blood were made available in the World War II European Theater of Operations. Comparative studies of acid-citrate-dextrose solutions (British and American) and Alsever's solution showed that:

- a. ACD solution was simpler to prepare and autoclave than Alsever's.
- b. Fibrin formation was minimal.
- c. When red cell survival studies were used, both ACD and Alsever's solution supported 70% cell survival during the first 48 hours after transfusion up to 15 days of storage.

As storage time was extended, ACD solution was accepted as the standard preservative based on posttransfusion survival. It was Loutit (1943) who studied the effect of acidification, in an attempt to resolve the problem of caramelization of the citrate-dextrose during autoclaving.

On 1 April 1945, 600 ml ACD bottles were substituted for the 1000 ml Alsever's solution. Later, Ross showed the same *in vivo* red cell survival of 120 ml ACD solution with 480 ml of blood, and 50 ml ACD solution with 450 ml of blood. Therefore, military blood logistics were improved by increased storage time and decreased bottle size. The ACD solution had a pH of 5.0 and contained 2.5 g disodium citrate and 3.0 g dextrose. The results, which were determined by the radioactive isotope technique, showed the *in vivo* survival of erythrocytes to be the same in both solutions.

*References immediately follow this section.

Military Blood Banks

In 1942, Walter Reed General Hospital established a blood bank and, by early 1944, blood transfusions were standard procedures in most general hospitals in the Zone of the Interior. At this time, Emerson and Ebert reported their studies on shock. Two concepts, generally accepted, concerning shock were: (1) that the pathogenesis of traumatic shock is a reduction in the circulating blood volume; and (2) that an essential feature of shock therapy is the correction of this deficiency by blood replacement. Emerson and Ebert found that:

a. The arterial blood pressure proved the most reliable clinical index to blood volume deficiency.

b. A normal hematocrit reading or the demonstration of only a mild anemia within a few hours after injury should not be interpreted to mean that severe blood loss had not occurred.

The case fatality rate for all casualties admitted in severe shock was 32%. When, however, the arterial pressure on admission exceeded 85 mm Hg, the case fatality rate was only 10%. The majority of deaths occurred in casualties with abdominal wounds.

Appointment of Consultant on Transfusion and Shock (15)

A consultant on transfusion and shock was even more necessary in the European than in the Mediterranean Theater, since several armies operated in it, with several widely separated blood bank units attached to them.

The question first came up on 2 January 1944, when COL James C. Kimbrough, MC (M.D.), was informed by COL Elliott C. Cutler, MC (M.D.), of the provisions for the whole blood service. It was pointed out to him that the highly specialized nature of this service made it essential that a competent officer be placed in charge of it. On 5 January, MG Paul R. Hawley, MC (M.D.), instructed COL James B. Mason, MC (M.D.), to appoint an officer to direct the whole blood service in the theater. It was highly desirable that he be appointed promptly, for basic decisions had already been taken about the service; a large quantity of equipment was already available; and personnel would soon be assigned. This was, therefore, the time for a director to take hold of the service and weld the separate parts into a whole. The officer nominated, General Hawley specified, must be a forceful executive, with a good knowledge of Army organization and operations, and must be qualified, from a professional standpoint, to advise on the use of whole blood.

COL Mason at once nominated CPT Robert C. Hardin, MC (M.D.) for the position, on the ground that he was better acquainted with all the details of the acquisition and processing of blood than any other officer in the theater. CPT Hardin was appointed theater transfusion officer on 7 February 1944.



Left to right: COL Elliott C. Cutler, MC (M.D.), LTC Ralph S. Muckenfuss, MC (M.D.), and MAJ Robert C. Hardin, MC (M.D.) (Summer 1944).

Korea

ACD-preserved blood in glass bottles was used on casualties during the Korean War. Among the conclusions reached is the following concerning blood transfusion:

Extensive experimental studies showed that there was no significant difference in the effectiveness of intra-arterial and intravenous administration of blood. It was concluded that it was the rate of transfusion, not the route, that was the important factor.

Other Observations During the Korean War

a. Group O, universal donor blood was available in Korea 12-14 days after collection.

- b. Massive and repeated transfusions were given.
- c. Samples from the blood bottle gave plasma hemoglobin recordings of 50 mg/100 ml ten days following collection, and 100 mg/100 ml on the 28th day.
- d. Abnormally high plasma potassium levels were not encountered after massive transfusions unless renal failure was also present.
- e. Osmotic fragility of red cells varied little during the first two weeks after collection, but rose sharply thereafter.
- f. Brief interruption of refrigeration caused irreversible changes in red blood cells. This was observed not via hemolysis in the bottle, but rather by poor *in vivo* survival following transfusion.
- g. Patients who received more than 15 pints of blood often showed a tendency to ooze from cut surfaces.

Plastic Blood Bags

Plastic blood bags were developed, but not used during the Korean War. Military hospitals were using plastic bags routinely by 1957, and civilian hospital blood banks adopted this procedure several years later.

Red Cell Preservation 1954-1973

In 1954, Valtis and Kennedy reported the increased affinity for oxygen by stored red cells. The two teams of Chanutin and Curnish and Benesch and Benesch reported on the role of 2,3-diphosphoglycerate (2,3-DPG) and the oxygen dissociation curve. That the oxygen dissociation curve is shifted to the left with preserved red cells which are low in DPG was reported by Åkerblom *et al* in 1968, and Bunn *et al* in 1969.

Gibson described a citrate-phosphate-dextrose (CPD) solution for the preservation of human blood in 1957.

Current Aspects of Blood Logistics

How far down the road of progress have we moved in military blood banking since World War II? It would seem that the refinement from the Korean War came in the use of plastic bags for blood collection. The integral donor tubing provided a closed system of collection and eliminated glass bottles and separate donor sets. The disposable nature of the plastic unit also eliminated a large incidence of pyrogen reactions experienced with the older rewashed donor sets. The logistic improvement was the achievement of a 75% reduction in the basic load provided in the Table of Organization and Equipment for bleeding teams.

Buffered Saline (Ringer's Lactate)

Several studies concerning the use of Ringer's lactate in hemorrhage and shock have been reported. In Vietnam, multiple casualties requiring blood transfusion were started in some emergency rooms on Ringer's lactate and later transfused with crossmatched blood. Some hospitals used group O universal donor blood immediately when faced with groups of casualties.

Brigadier General Thomas J. Whelan, Jr., MC (M.D.), in presenting the paper "The Interdependence of Military and Civilian Surgery," commented on blood banking in Vietnam. He noted that since 1967 the majority of blood for Vietnam has been drawn in the United States. Resuscitation procedures have been influenced by advances in technics developed by civilian surgeons. The difference in resuscitation procedures, as practiced in World War II, the Korean War, and the Vietnam Conflict, reflects this fact. A severely wounded individual will have an endotracheal tube inserted and will have air or oxygen administered under pressure first from an Ambu bag and shortly from a volume-controlled ventilator. An arterial blood sample will be taken for pH, pO₂, and pCO₂; a central venous catheter through a percutaneous subclavian vein approach will be placed for administration of large volumes of blood.

Early in the campaign large amounts of Ringer's lactate were administered, influenced by contemporary studies during 1965 to 1968. Fluid overload resulted from overzealous use of this fluid and exacerbated problems of "wet lung." Central venous pressure was a poor indicator of this overload since diffusion of balanced salt solutions to all body fluid compartments caused only late changes in the central venous pressure. As soon as blood gas analyses were possible in Vietnam in 1967, early mistakes in giving empiric sodium bicarbonate were corrected. Upon arrival at the hospital, many patients were actually alkalotic from the hyperventilation of pain and anxiety; only the patient in deep and prolonged shock was acidotic. Even in these latter patients sodium bicarbonate only helped temporarily by neutralizing lacticacidemia. Improvement of tissue perfusion by replacement of blood volume proved to be the only permanent measure.

In the Vietnam Conflict, operation for the control of hemorrhage as a vital part of resuscitation was practiced much more frequently than in other wars. The reason for this modification of approach was the early arrival of severely injured patients at the hospital, made possible by the extensive use of helicopter evacuation direct from the battlefield to the hospital.

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INTRODUCTION

During 13 years of the past three decades, well over 15,000,000 units of human blood and blood components have been collected, processed, and much of it shipped to areas of military exigency occurring outside the Continental United States. Upon arrival, the whole blood was stored under refrigeration, sometimes reprocessed and reshipped to medical teams and hospitals within the area of combat operations. At its final destination, the blood was used either as group O universal donor blood or was crossmatched with the wounded recipient soldier. Much medical data have been accumulated during these combat experiences, including the role of blood transfusion in resuscitating the slightly, moderately, and severely wounded soldier (1). The World War II and Korean War programs have been well described and evaluated, but it seems quite proper to study them again, as we now place the Vietnam blood program experience in retrospect.

In making the analysis of three large military blood programs, we have confined ourselves to an outline protocol, made purposely narrow

for two reasons. First, we decided to address only the major areas which would be applicable to civil disaster programs; and, second, it is not the purpose of this article to present an historic document, because this is the mission of The Historical Unit, United States Army Medical Department. The outline protocol follows:

1. Collection, processing, shipping, storage, and usage of blood.
2. Patient identification.
3. Sample identification.
4. Crossmatch procedures.
5. Use of group-specific blood.
6. Use of group O "universal donor" blood.
7. Transfusion reactions:
 - a. Prevention.
 - b. Detection.
 - c. Management.
8. Frozen blood.
9. Blood components.
10. Special problems for future resolution:
 - a. Need for more efficient instrumentation.
 - b. Other problems.
11. Discussion for areas pertaining to civil disasters:
 - a. Use of A and O blood.
 - b. Patient triage and communication.
 - c. Training programs.
12. Recommendations for civil disasters.

Running throughout the analysis certain webs of truth are continually entwining and eventually they are appreciated by their obviousness. The truths which evolve are on both the positive and negative sides of the goal of preventing injury to the recipient from blood transfusion.

COLLECTION, PROCESSING, SHIPPING, AND STORAGE OF BLOOD (2-4)

In Tables 1 and 2, the shipment of group O blood is shown with the heavy usage in Europe occurring in the period April 1944-June 1945.

TABLE 1

WORLD WAR II BLOOD PROGRAM

	<u>Total Collections*</u>	<u>Shipped to Europe**</u>	<u>Shipped to Pacific**</u>	<u>Military Hospitals in U. S. ***</u>
American National Red Cross	13,326,242	205,907	181,555	310,135
ETOUSA# Blood Bank	130,635##			

*4 Feb 1941 - 15 Sep 1945.

**Group O blood.

***All blood groups, plasma and whole blood.

#European Theater of Operations, U. S. Army.

##April 1944 - June 1945, essentially group O blood.

Relatively little of the total collection was used as whole blood.

TABLE 2

DELIVERY OF WHOLE BLOOD TO USING HOSPITALS, ETOUSA

April 1944 - June 1945

<u>Communications Zone</u>	<u>U. S. Army Zone*</u>	<u>Total**</u>
50,561	266,238	316,799

*Only group O blood used in combat zone.

**Total loss less than 15%.

This was the first large-scale use of whole blood in combat history.

The usage rate shown in Table 3 is 1.33 units of blood to each of the wounded recipients.

TABLE 3

**RATIO OF BLOOD DELIVERED TO ADMISSIONS OF WOUNDED
AT FORWARD HOSPITALS, ALL U. S. ARMIES, ETOUSA**

June 1944 - May 1945

<u>Admissions</u>	<u>Pints of Blood Delivered</u>	<u>Ratio of Blood to Wounded</u>
340,351	266,238	1:1.33

In comparison to Korea and Vietnam, the 1:1.33 ratio of blood to wounded is conservative usage.

One certainly may wonder about the great difference between the 13 million units of blood collected and the 697,597 units shipped to military installations. The dried plasma program required 10,299,470 pints of blood for processing 3,147,744 (250 ml) and 3,049,636 (500 ml) packages of plasma. Albumin production as of 1 September 1945 required 2,329,175 pints of blood to produce 569,014 packages of albumin. These were delivered to the US Naval Medical Supply Depot. One unit of albumin consisted of 100 ml of a 25% solution. The maximum recommended in 48 hours for one patient was 10 units, or 250 g.

The risk of serum hepatitis from use of dried blood plasma was not well known in January 1944. This complicated the disposition of surplus stocks and led to the replacement of plasma in the Korean War by serum albumin.

STORAGE AND SHIPPING

General Kendrick noted that in the European Theater, Medical Supply provided storage facilities and transportation, but the real responsibility for handling this perishable item, that could be lethal without proper supervision, belonged to the transfusion service operating under the overall direction of the theater blood bank. It is unfortunate that the same policy was not employed in the Korean War.

TRANSFUSION REACTIONS - WORLD WAR II

Table 4 shows the transfusion reaction rate to be 1.7% in a study of 42,689 transfusions of principally group O blood. There were 152 allergic and 500 pyrogenic reactions. It was noted that blood shipped from the United States accounted for the largest proportion of allergic reactions, but by far the lowest proportion of pyrogenic and hemolytic reactions. *Many pyrogenic reactions were due to reused blood donor and recipient sets.* There were 64 hemolytic reactions, with 33 deaths. This is the area where analysis is needed. What caused fatal reactions from group O blood used as universal donor blood to A, B, AB, and O recipients?

TABLE 4
TRANSFUSION REACTIONS IN ETOUSA

	<u>Transfusions</u>	<u>Reactions</u>	<u>Classification</u>			
			<u>Allergic</u>	<u>Pyrogenic</u>	<u>Hemolytic</u>	<u>Deaths</u>
Field Hospitals (17 Weeks)	17,769	224	52	160	12	6
Evacuation Hospitals (20 Weeks)	<u>24,920</u>	<u>500</u>	<u>100</u>	<u>340</u>	<u>52</u>	<u>27</u>
	42,689	724*	152	500	64	33

ETOUSA = European Theater of Operations, U. S. Army

*Transfusion reaction rate = 1.7%

The pyrogenic reactions can be readily attributed to reusable, rubber donor and recipient sets used with glass bottles. Later, disposable plastic donor and recipient sets practically eliminated this problem.

PROBLEM AREAS

1. Mislabeled blood.
2. Wrong pilot tubes.
3. Contaminated blood.
4. High titer isoagglutinins A or B.

5. Immune A or B.

6. Hemolysins A or B.

Blood drawn from military personnel in Europe who had received immunization for cholera, typhoid, paratyphoid, typhus, etc., may have had immune A and B and hemolysins. Tests for these antigen-antibody systems were not employed. Screening for isoagglutinins A and B was performed using saline suspensions of A and B red blood cells. If the cells were antigenically weak, such as A₂, or if the suspensions of cells were used repeatedly for 6-8 days, cutoff titers such as 1:200; 1:256, etc., would have been meaningless. Therefore, any or all six points listed in the problem areas could have occurred.

KOREAN BLOOD PROGRAM (2)

Table 5 shows that 340,427 pints of group O, Rh positive blood with A and B isoagglutinin titers of less than 1:256 were shipped to Korea during the period 1950-1953. The units of blood per casualty transfused averaged 1.95 with a high of 2.54.

TABLE 5

KOREAN WAR BLOOD PROGRAM

25 June 1950 - 25 July 1953

	<u>Received from American National Red Cross</u>	<u>Shipped to Korea* Via Japan (406th MGL)</u>
Armed Services Whole Blood Processing Laboratory, Travis AFB, California	397,711	340,427
	Collected in Japan	
406th Medical 1951	38,772	
General Laboratory, 1952	34,503	
Tokyo, Japan	<u>73,275**</u>	

*Group O Rh pos., A and B isoagglutinin titer, less than 1:256.

**Used in U. S. hospitals in Japan; shipped to Korea as needed.

There are no official reports of death attributable to hemolytic transfusion reaction. However, Crosby noted that after massive transfusions of group O blood to recipients who were A, B, or AB, severe reactions occurred when type-specific blood was given. Therefore, it was recommended that when five or more units of group O blood were given

to a patient of groups A, B, or AB, no attempt should be made to transfuse with the hereditary blood group within 14 days. If more blood is needed, the patient should receive group O blood. In Korea, the use of group O blood with A and B isoagglutinins titered below 1:200 proved a safe practice (5).

On the basis of the observations of the Surgical Research Team, it was concluded that the incidence of incompatible transfusion reactions in Korea was exceptionally low. The statistics of the Renal Treatment Center support this. Over 50,000 transfusions were given in Korea in 1952. In that year, only four patients were admitted to the Center with acute renal insufficiency due to an incompatible transfusion. There probably were more than four reactions: some died and some recovered without going to the Renal Treatment Center at Wonju. Inquiries found few of them (5).

Although the rate of incompatible transfusion reactions was low, there are no accurate figures on the subject. It is important to have a continual examination of the problem. The careful study of each reaction provides information that is needed to prevent subsequent reactions. Even the meager information provided by the statistics of the Renal Treatment Center suggested that most, if not all, reactions in Korea were the result of using locally procured blood. To the end of obtaining this essential information, two recommendations were made:

1. The personnel who administer transfusions should be well trained to recognize reactions and should know what records to make and what specimens to collect so that the cause of the reaction can be identified.
2. There should be quickly available in the combat zone an expert who can interpret the evidence and identify the cause of the reaction (2,6).

SHELF LIFE OF BLOOD - KOREA

The average remaining shelf life of whole blood reaching Korea was 9.4 days. Some forward units reported that blood being used was 9 to 20 days old.

PLASMA AND HEPATITIS

Late in 1951 in Korea, the incidence of hepatitis after plasma transfusion reached 21%, in sharp contrast to the reported World War II incidence of 7.5%. Improved laboratory procedures could account, at least in part, for the higher incidence. Plasma was used in Korea initially by the Army until the high incidence of serum hepatitis made it necessary to discontinue its use; serum albumin was substituted.

VIETNAM (7)

As of 31 December 1970, over 2 million and a half pints of blood had been sent to Vietnam from the United States, Okinawa, and Japan (Table 6). Blood drawn in collection centers throughout the United

TABLE 6

VIETNAM BLOOD PROGRAM

	<u>Blood Available in Vietnam</u>	<u>Units Transfused</u>	<u>No. Patients Transfused</u>	<u>UD Transfusions</u>	<u>HTR</u>	<u>SUT</u>	<u>FFP</u>	<u>Broken Units</u>
1967	289,286	101,103	20,591	1,918	17	1,053	0	0
1968	476,330	175,810	40,842	25,358	17	1,723	600	29
1969	470,481	164,157	35,841	54,126	12	3,670	3,338	433
1970	267,015	116,418	30,707	19,017	2	NR	2,054	581
	1,503,112	557,488	127,981	100,419	49	6,446	5,992	1,043

UD = Universal Donor
HTR = Hemolytic Transfusion Reaction (9 deaths)
SUT = Single Unit Transfusion
FFP = Fresh Frozen Plasma
U/P = 4.4 Units/Patient

States was shipped to the Armed Services Whole Blood Processing Laboratory at McGuire Air Force Base and then flown by C141 Starlifter to Vietnam via Japan (Fig. 1). Once the blood was "in country," distribution

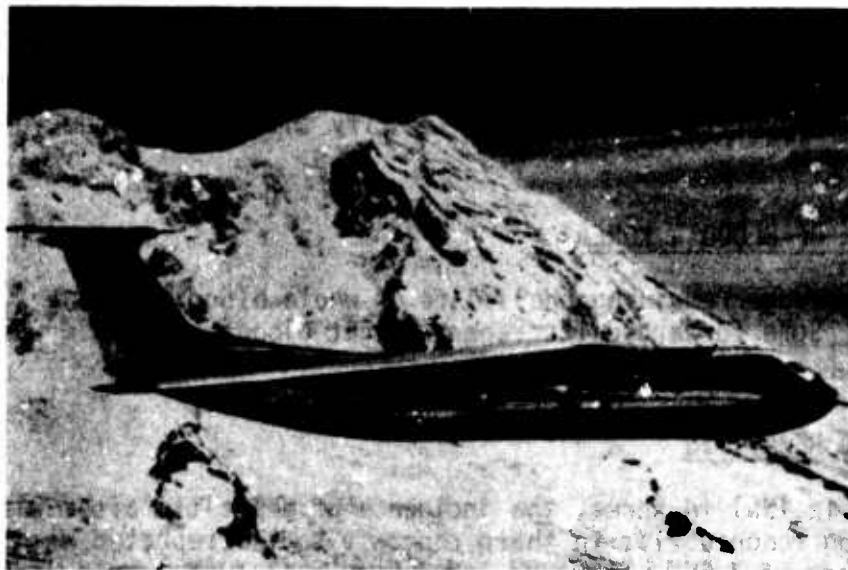


Fig. 1. The Air Force Starlifter C-141 delivered blood shipments on time halfway around the world.

was made by helicopter throughout Vietnam (Fig. 2). Figures 2a and b show an increase in combat activity until the entire area was enveloped.

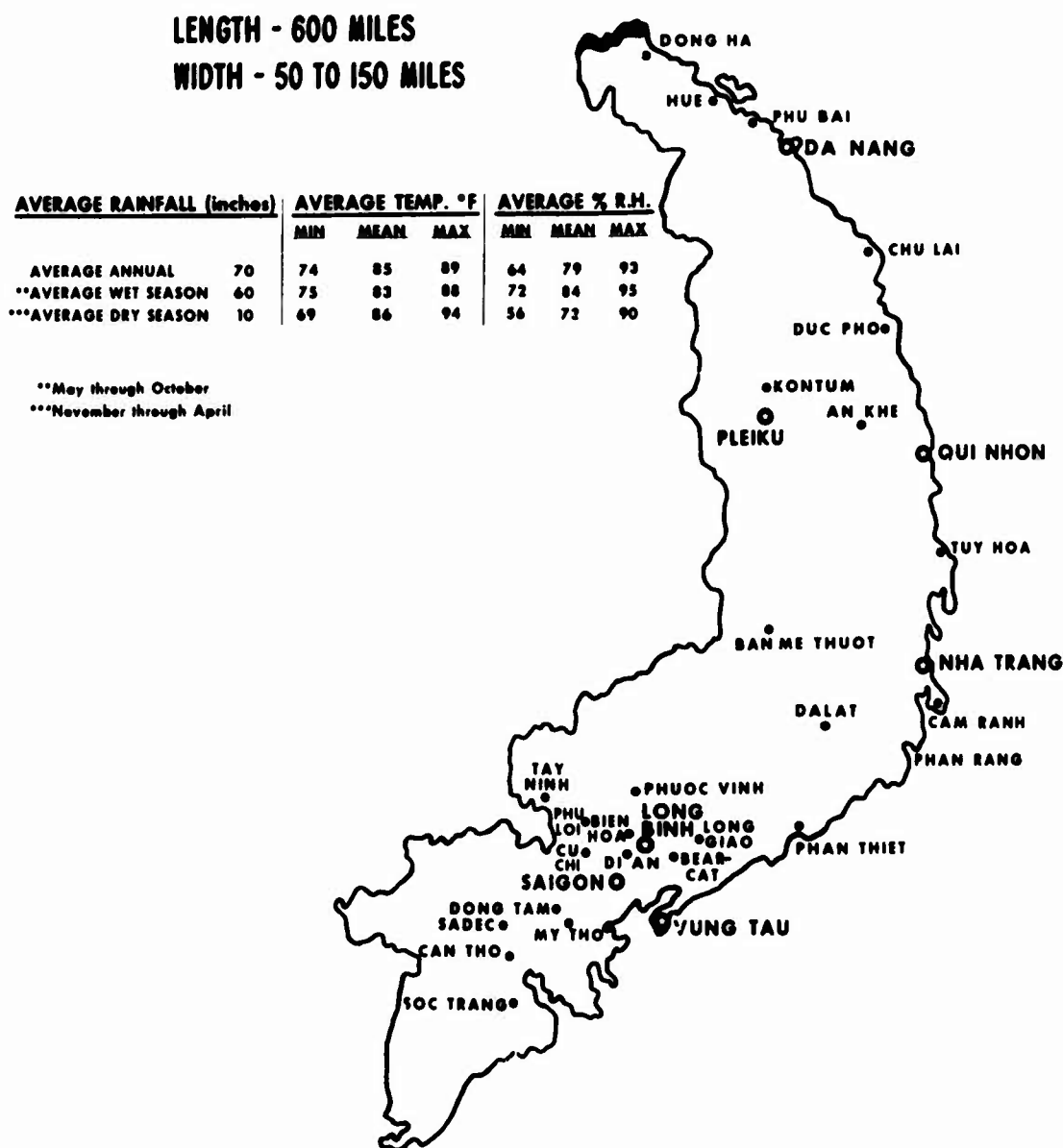


Fig. 2. Blood was distributed and maintained in constant supply throughout Vietnam. The area of nonactivity for 6 months or more could become a combat area overnight; hence, the need to maintain blood storage throughout Vietnam.

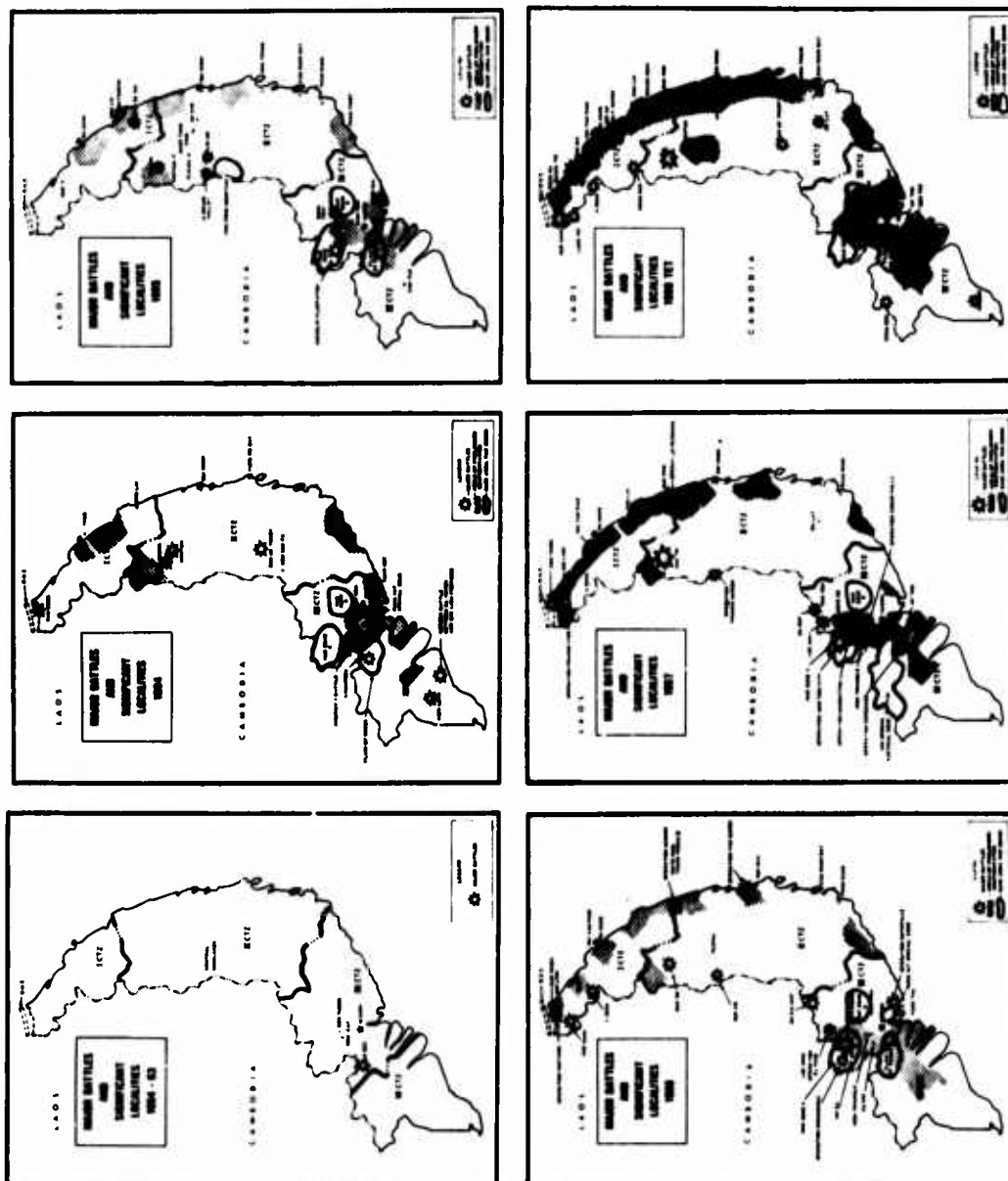


Fig. 2a. The composite maps of Vietnam show that combat activity increased steadily until virtually all of the country was involved.

TROOP LEVELS IN VIETNAM

JUL 1965	75,000
JAN 1966	250,000
NOV 1966	389,000
NOV 1967	525,000
JUL 1968	525,000
JUL 1969	500,000
JAN 1970	499,000
APR 1970	349,000

Fig. 2b. Combat activity and blood usage were reflected in the troop levels to some extent.

This must be kept in mind when examining the blood distribution and supply of blood maintained constantly throughout all medical installations in Vietnam. The sporadic and unexpected Viet Cong attacks required a local blood supply much as communities insure against disaster with fire and police departments. Criticism of this policy on any particular Monday morning, after the fact, is just not valid. The breakdown of blood groups is shown in Table 7; an unusually large amount of group B was supplied because of the high frequency of the B gene in our wounded Asiatic allies.

Table 8 shows the amount of blood drawn in Vietnam and on ships patrolling the offshore areas. Blood collection in Vietnam was discouraged due to hepatitis and malaria. Much of it, however, was drawn to provide fresh blood for coagulation problems. This was alleviated somewhat starting in 1968 when fresh frozen plasma from Japan and, later, CONUS was provided and this supply was sustained. Shipment of the fragile units was

TABLE 7
CONUS BLOOD PROGRAM FOR VIETNAM

1966 - 1971

O - 646,299
B - 132,746
A - 261,297
AB - 11,182

TOTAL 1,051,524

The requirement for group B was unusually high due to the high frequency of the B gene in our wounded Asiatic allies.

TABLE 8
BLOOD DRAWN IN VIETNAM AND ON SHIPS

1967	-	6,561
1968	-	6,213
1969	-	5,781
1970	-	6,157

TOTAL 24,712

The blood drawn on ships was probably the safest and best source of fresh whole blood.

made possible by using Aircap[®]* material for packaging (Figs. 3 and 3a). This is the first time that this component with coagulation factors, including V and VIII, has been used on a large scale in combat.

* Sealed Air Corporation, 179 Gaffle Road, Hawthorne, New Jersey 07506.

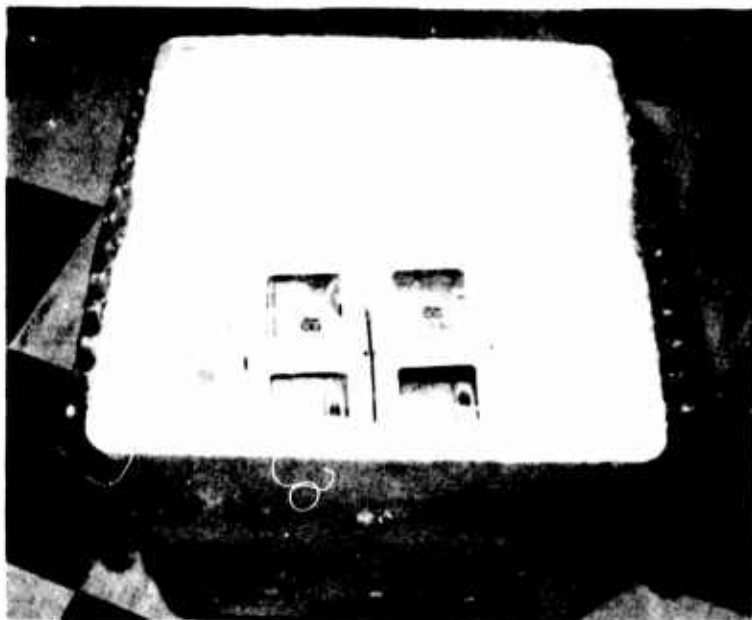


Fig. 3. Aircap provided the answer to shipping fresh frozen plasma and cryoprecipitate long distances without breakage.



Fig. 3a. This mode of shipping permitted the first large-scale use of Factor VIII component in a combat zone.

Table 9 shows the distribution of blood groups sent to Vietnam starting initially with group O in 1966; by 1970 an ample supply of all blood groups was made available.

TABLE 9

CONUS BLOOD PROGRAM FOR VIETNAM

<u>1966</u>	<u>1967</u>	<u>1968</u>	<u>1969</u>	<u>1970</u>	<u>1971</u>
O - 14,915	O - 82,878	O - 199,900	O - 200,601	O - 104,269	O - 43,736
B - 154	B - 20,945	B - 34,262	B - 37,962	B - 26,049	B - 13,374
A - NONE	A - 11,575	A - 82,512	A - 89,934	A - 52,184	A - 25,092
AB - NONE	AB - NONE	AB - NONE	AB - 3,671	AB - 4,694	AB - 2,817
TOTAL 15,069	115,398	316,674	332,168	187,196	85,019

The table shows the gradual increase in supply until ample supplies of all blood groups were provided on a sustained basis.

Referring to Table 6, it can be seen that 37% of the blood supplied was actually used. There were 127,981 patients transfused for an average of 4.4 units of blood per casualty transfused. This represents a continued increase in combat transfusion therapy as noted in Table 10.

TABLE 10

BLOOD USAGE PER CASUALTY*

<u>World War II</u>	<u>Korean War</u>	<u>Vietnam</u>
0.9 to 1.1	1.9 to 5.5	4.0 to 5.0
avg 1 unit/ casualty	avg 2 units/ casualty	avg 4.4 units/ casualty

*Issued as glass bottles or plastic bags equivalent to 500 ml of whole blood.

The dramatic increase in blood therapy is seen over a three decade span in combat medical treatment.

UNIVERSAL DONOR TRANSFUSIONS (9)

The Vietnam blood program provided the opportunity for the first large-scale use of specific blood groups in association with sophisticated crossmatch procedures for serological compatibility. It should be noted that 100,419 universal donor transfusions were given without a single reported transfusion death from incompatibility (Table 6). *On the other hand, nine reported deaths were attributed to hemolytic transfusion reaction following extensive crossmatched blood compatibility testing.* The underlying cause of this enigma is simple. During mass casualty situations resulting from combat, a hospital receiving ten, 20, or 30 casualties had to make a decision to use group O universal donor blood or group specific, crossmatched blood. Those choosing the latter plan ran the whole gamut of possible errors:

1. Patient identification.
2. Blood sample identification.
3. Crossmatch procedure time.
4. Release of blood.
5. Transfusion of correct recipient.

What might have happened was that blood crossmatched for one person with a resulting compatible crossmatch (including the antiglobulin Coombs test) was then, inadvertently, given to another patient, sometimes with a fatal outcome.

Special Case Report

A patient with multiple fractures of the right arm and forearm was given one unit of O NEG "high titer" blood at a clearing station. The patient was evacuated to the 8th Field Hospital where, of five units of A NEG blood crossmatched prior to surgery, only two were compatible. The patient's serum contained free hemoglobin and the direct Coombs test was positive. Following amputation of the forearm he was given only O NEG packed cells. Subsequent anti-A titers of the original O NEG unit in saline and Coombs were 1:256 and 1:32,768, respectively. Anti-B titers were also elevated. After two days of oliguria the patient ceased to hemolyze and experienced a return to normal renal function. He was evacuated on the seventh hospital day.

In Crosby's Korean War studies, as noted earlier, the recommendation was made that when five or more units of group O blood are given to a patient with group A, B, or AB, there should be no attempt to transfuse with the hereditary blood group within 14 days. When the patient requires more blood, group O blood should be used.

Patients received at CONUS hospitals following multiple transfusions of group O universal donor blood showed a variation in persistence of clinically significant transfused antibody. Barnes and Allen (8), using several serologic techniques, were able to revert back to the patient's hereditary blood group when no transfused antibody was demonstrable. Sometimes this was possible 12 days following multiple transfusions of universal donor blood. On the other hand, the patient continued to receive group O packed cells if residual transfused antibody was detected.

ISOAGGLUTININS A AND B (10-15)

During the Korean War, 55-65% of group O donors could be classified as low titer anti-A and anti-B (< 1:200).

During the Vietnam Conflict, this percentage varied due to very specific reasons. A study in 1965 at Fort Benning revealed that immunizations can cause significant antibody responses.

Table 11 shows the results of the testing. Fifteen percent had a saline anti-A titer in excess of 1:200. Seventy percent had a hemolysin and 38% had immune A or B antibodies in titer > 1:20. The immune response was caused by plague vaccine which had approximately 53 µg/ml of A substance.

TABLE 11

ANTIBODY INCIDENCE - FORT BENNING *

	PRE VIETNAM	FROZEN	POST VIETNAM
NATURAL A			
1:200	15.2	16.1	62.5
HEMOLYSIN	70.0	-	77.0
IMMUNE			
OVER 1:20	38.0	67.0	70.0

***OVER 2, LESS THAN 5 IMMUNIZATIONS**

The loss of group O universal donors due to plague immunizations containing A substance was discovered by the Fort Knox blood research field team at Fort Benning in 1965.

Table 12 gives the results of another study at Fort Knox, involving group 0 recruits before and after one and two plague immunizations. It

TABLE 12

ANTIBODY INCIDENCE - FORT KNOX

	INDUCTEE WITHOUT IMMUNITY	RECRUIT PLAGUE			FORT KNOX TROOPS
		0	1	2	
NATURAL A 1:200	17	20	51	70	32
HEMOLYSIN	7	41	64	80	64
IMMUNE OVER 1:20	13	7	27	30	6

Additional control studies were conducted at Fort Knox to confirm the Fort Benning finding.

can be seen that anti-A in excess of 1:200 rose from 20% to 70% and immune anti-A > 1:20 increased from 7% to 30%. Plague vaccine was later produced without the A substance thus obviating the problem. Several methods for preparing the group 0 serum-saline dilutions were used. Figure 4 shows an accurately calibrated loop which delivers .01 ml of serum. Another method of determining the isoagglutinin titer is shown in Figure 5. This is a 10 λ capillary tube and is still used for titering serum of group 0 donors.

During the period 1965-1966 when plague vaccine was causing an increase in anti-A and anti-B titers, group 0 universal donors were obtained from military recruits prior to any immunizations.



Fig. 4. This method, devised by J. R. Brewer, J. E. Rogers, Jr., and J. E. Spiker, Jr., during their Blood Bank Fellowship training, was used during the Fort Benning study to screen group O sera for A and B isoantibodies.



Fig. 5. This method of titrating O sera for A and B isoantibodies was developed at Fort Knox in 1966.

Tables 13a and b give an example of what the yield of group O universal donors would be in 1971.

TABLE 13a

Yield of Group O Universal Donors in 1971 (Prior to Immunization)

Isoagglutinins A and B < 1:200	over 90%
Immune A and B < 1:20	71 - 87%
Hemolysins A and B (immediate spin)	94 - 99%

TABLE 13b

Yield of Group O Universal Donors in 1971 (Following Immunization)

Isoagglutinins A and B < 1:200	80%
Immune A and B < 1:20	80%
Hemolysins A and B	90%

If one applies all three criteria in selection of group O donors in Table 13a, the yield of low titer O's would be 80%. The yield in Table 13b following immunization would drop to 62%!

QUESTIONNAIRE ON COMPATIBILITY TESTING AND RESULTS

In 1970, a questionnaire was mailed to 50 authorities in the field of blood grouping, blood banking, and blood transfusion. The questions raised pertained to the level of technical proficiency of the individual performing the serological crossmatch: (1) should one or two technicians do the testing (blind) and correlate results; (2) where did responsibility begin in the blood bank; and (3) who should sign the crossmatch certifying compatibility?

The results revealed that a majority of those polled were in agreement on the following: that the chief of the laboratory or pathology service, under whom the blood bank operates, is responsible for all

phases of compatibility testing and delegates authority to a technician or technologist to perform the testing and to sign the crossmatch forms certifying compatibility. They also felt that requiring an MT(ASCP)BB in all blood banks was too stringent and not possible (lack of certified personnel) at that time (1970). Two technicians or technologists performing the testing blind and later correlation done by a third individual was considered to be fine, but not always possible. Therefore, a highly qualified individual considered fully competent by the pathologist could suffice in many small facilities. Finally, many agreed the crossmatch forms were in need of revision in the immediate future.

HEPATITIS IN VIETNAM (See Annex M)

The incidence rates of viral hepatitis in US Armed Forces stationed in the Republic of Vietnam (RVN) are given in the following table as rates per 1,000 average strength per year.

1965	5.70
1966	3.99
1967	7.01
1968	8.59
1969	6.41
1970	7.24

There are no figures available on the number of cases of hepatitis arising from blood transfusion therapy. The blood sent to Vietnam, however, would be expected to have an incidence of Australia-antigen (Au), also called hepatitis associated antigen (HAA), no greater than one positive unit for every thousand pints of blood sent from CONUS.

In a preliminary study, the incidence of Australia antigen (Au) and/or antibody (anti-Au) in soldiers returned from Vietnam was found to be approximately 18 positive tests per 1,000 returnees (16).

THE VIETNAM FROZEN RED BLOOD CELL PROGRAM (17,18)

In 1966, the US Navy began to conduct feasibility studies in Vietnam to evaluate a frozen red blood cell blood bank in the combat zone. The purpose was to increase the capability and flexibility of the standard military blood bank supporting combat operations. Following careful serologic screening, special cells were chosen, drawn, and frozen. These included group O r/r, Kell negative, and Duffy negative; also group O r'/R₁, Kell negative, and Duffy negative. The Huggins freezing technique was used. Early conclusions based on 3,000 transfusions of frozen red blood cells to wounded servicemen in a combat zone are that there is technical feasibility as well as clinical acceptability in the operation of a frozen red blood cell blood bank system.

REFERENCE AND FORENSIC TESTING LABORATORY

The Fort Knox Reference and Forensic Testing Laboratory tested many bones for A and B blood group substance to assist the US Army Mortuary, Saigon, in the identification of human remains retrieved from helicopter and airplane crashes.

BLOOD PRESERVATION (19,20)

During the period November 1968 to March 1969, whole blood drawn at Fort Knox in acid-citrate-dextrose (ACD) and also ACD with adenine was shipped to Vietnam; 4,339 units, half with adenine, the other half without were shipped. Adenine was used to extend the shelf life of whole blood from 21 to 42 days and produced no adverse reactions; however, it was used prior to the 21-day expiration allowed for whole blood drawn in ACD.

FIELD REPORTS

Whereas an excellent job was done in reporting events occurring in the field concerning blood usage, hospitals not reporting blood transfused could account for 30% greater usage than officially reported (1970-1971). This probably applies to all areas of blood banking starting with the first shipment of 2,034 units whole group O blood from CONUS to the 406th Medical Laboratory on 15 June 1966.

CONUS-VIETNAM BLOOD PROGRAM

Annex F lists the active military blood donor centers (21).

TRANSFUSION REACTIONS IN COMBAT TREATMENT CENTERS

Table 14, Transfusion Reactions in Combat Treatment Centers, has considerable information for analysis. We have discussed the World War II and Korean War statistics on transfusion reactions in detail.

The Vietnam experience should have provided much more pertinent information because *emergency measures* of blood therapy were employed, with technics similar to those in use today in hospitals (civilian and military) throughout the United States. It is quite apparent that those hospitals receiving multiple casualties and treating them initially with group O universal donor blood avoided hemolytic transfusion reactions. On the other hand, those hospitals receiving similar numbers of casualties and attempting specific group blood therapy involving a crossmatch and a Coombs test, ran into significant difficulty resulting in hemolytic transfusion reactions. Some hospitals circumvented the problem of group O universal donor blood versus group specific crossmatched blood for mass casualty situations by using Ringer's lactate and following this with crossmatched blood.*

* See Historic Background References 2, 6, and 20 (pages 6 and 7).

TABLE 14

TRANSFUSION REACTIONS IN COMBAT TREATMENT CENTERS

	<u>Hemolytic Transfu- sion Reactions (HTR)</u>	<u>Other *</u>
1. World War II	33	652
2. Korean War	0	19
3. Vietnam Conflict	9	1,160

1. Study of 42,689 transfusions:

a. HTR deaths would extrapolate to 249 in 316,000 transfusions at a ratio of 1 pint of blood per wounded.

b. Other reactions would extrapolate to 4,890 using the 1 to 1 ratio of blood to wounded.

2. No hemolytic transfusion reactions reported as resulting in death 1950-1953. Nineteen other reactions reported in study of 757 patients. This would extrapolate to 6,984 in 291,000 transfusions at a ratio of 1 unit per wounded, or 3,480 in 145,000 transfusions at the ratio of 2 units per wounded, 1951-1952.

3. Based on 127,981 patients transfused with 557,488 pints of blood, January 1967 - December 1970.

* Transfusion reactions classified as allergic, pyrogenic, bacteriogenic; also described by chills, fever, urticaria, nausea, etc.

REDUCTION OF PYROGENIC, ALLERGIC, AND OTHER FEBRILE REACTIONS - AN APPROACH (21a,b,c,d)

Table 14 reveals a high percentage of reactions, other than hemolytic, occurring in combat casualties, especially those receiving multiple transfusions. Young males (18-25 years old) represent a large proportion of this group of reactions.

What major areas can one examine to determine how best to reduce or eliminate these categories of reactions?

1. Determination of whether reactions were related to transfusion or were the symptoms of the underlying condition (e.g., infection).

2. Collection of blood improperly resulting in contamination.

3. Improper storage and handling - faulty refrigeration.

4. Whether there was an accurate description of the symptoms of the reaction and follow-up studies. Assuming that red cell antibodies were looked for and not found, and that the patient was not already febrile, a febrile response to transfusions in civilian practice is most

commonly caused by white cell antibodies--but white cell antibodies (for all practical purposes) *are not found in previously untransfused males!* Therefore, concern over the HL-A antigens is apparently not a major requirement.

5. Febrile reactions can also occur because of antiserum protein (anti-IgA antibodies). A certain proportion of the population, not statistically ascertained, have an absence of IgA (immunoglobulin) in their serum. These persons will almost invariably develop anti-IgA when transfused or immunized with gamma globulin. Upon subsequent transfusion of whole blood, they are at a high risk of having an anaphylactoid transfusion reaction. An initial base line study is needed to determine the quantitative level of IgA in the serum and, if IgA is absent, it would be advisable to go further to determine if there were antibody against IgA in the serum.

6. Removal of buffy coat and platelet-poor packed red cells decreases the problem of sensitization or challenging dose from these elements (Figs. 5a and b).

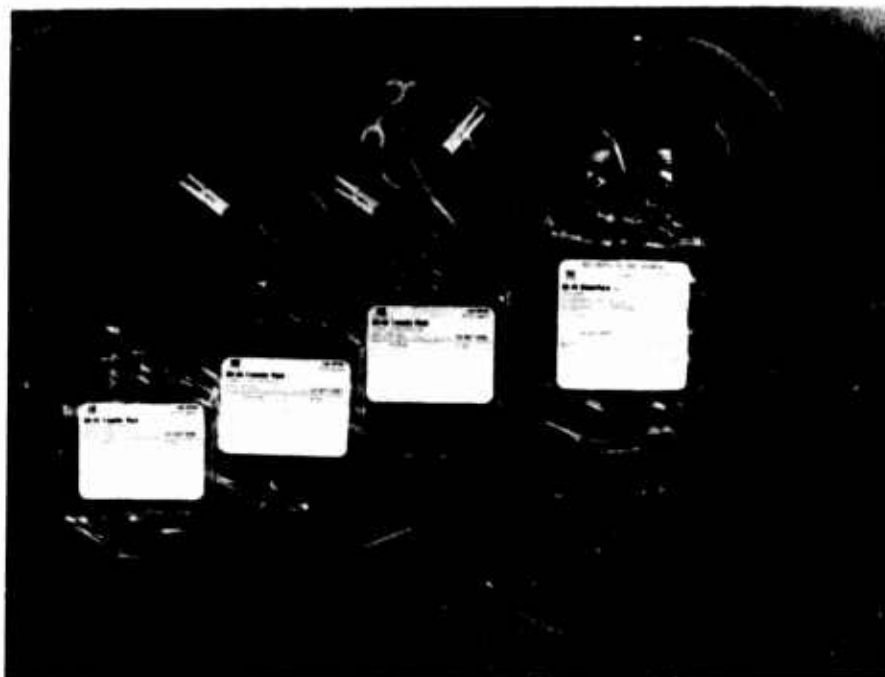


Fig. 5a. Offers opportunity to harvest packed red cells in the main container, platelet concentrate in a sterile attached pack, an AHF concentrate in an attached sterile pack and residual plasma in a sterile attached pack for further manufacture into albumin, fibrinogen, and gamma globulin.



Fig. 5b. This pack adds to the capability of the quadruple pack with the additional harvest of white cells in a 150 ml attached satellite pack.

7. The use of frozen red cells might prevent reactions if they were due to white cells or donor immunoglobulins. However, many of these reactions can be mitigated or prevented by measures short of using frozen blood: packed red cells or washed red cells, for example.

What appears to be emerging is the possible application of current blood component preparation and therapy, at least in part, to support blood logistics normally using whole blood and plasma expanders. The use of the ABO blood groups and universal donor group O blood would hopefully be *augmented* in future military exigencies and/or civil disasters with various blood components and frozen red cells. The newer technics just discussed would certainly be available in the larger treatment centers for men who have manifested transfusion reactions described in this section.

The usefulness of frozen red cells for rare donor groups is quite feasible; certainly, it can be considered for the peaks and valleys of blood surplus and shortage that face both civilian and military operations. In short frozen red cells provide a "backup" blood supply and this would have been much appreciated during the 1968 TET offensive in

Southeast Asia. Valeri and Zaroulis* report the incubation of red cells that had been stored at 4 C for 21 to 28 days with a solution containing pyruvate, inosine, phosphate, and glucose, restored the red cell oxygen transport function to normal and improved the 24-hour posttransfusion survival. The washing procedure removed, in addition to glycerol, the products of hemolysis, the potentially toxic materials used for rejuvenation, the anticoagulant preservative, isoagglutinins, protein and non-protein plasma factors, and a large part of the white cells and platelets. Application of this technic in blood logistics is presented in the other sources.**

CONUS STUDY OF TRANSFUSION REACTIONS (22)

Since June 1968, data have been collected concerning the numbers of blood transfusions and transfusion reactions occurring in military hospitals in the First US Army area. Analysis reveals that in excess of 2,000 units of whole blood and blood components were transfused to patients in these medical facilities. There were 253 reactions reported in three classifications: (1) febrile reactions - 72; (2) urticarial reactions - 178; (3) hemolytic reactions - 3. No deaths from blood transfusion reaction were reported.

One unit of blood was transfused for every four units crossmatched in the blood banks of the various hospital pathology services, representing 20,000 transfusions actually used of the 80,000 units crossmatched for serologic compatibility. Remaining time of the 21-day shelf life of whole blood allowed many of the blood units to be re-crossmatched for other patients. On the other hand, blood continually committed to patients by crossmatch and not used resulted in outdating and loss of the blood.

BLOOD THERAPY TREND

The use of blood components has increased sharply, especially in the larger hospitals and this upward trend continues. The greater use of blood components is the result of newer, more established medical treatment practices. With the shift in emphasis away from whole blood, the result is the extension of one unit of whole blood into two, three, and four useful products. Eventually, blood wastage from outdating will be reduced sharply.

DISCUSSION (2,23-32,56)

Special problems related to blood and blood component usage resulting from small and large civil disaster situations continue to face

* See Historic Background Reference 21 (page 7).

** See Historic Background Reference 3 (page 6).

medical installations and, in particular, the blood bank staff. Much has been learned from the first large-scale use of blood therapy in World War II, the Korean War, and, again, in the Vietnam Conflict. Some of the advancements include:

1. Standardization of ABO blood group nomenclature.
2. Improvement of blood preservation solutions.
3. Role and limitations of plasma in resuscitation.
4. Improvement of blood collection (plastic bag) unit.
5. Detection of A and B blood group substance in certain biologics:
 - a. Influenza vaccine.
 - b. Plague vaccine.
 - c. Placental gamma globulin.
6. Use of frozen blood and blood components (fresh frozen plasma and cryoprecipitates) in the combat zone.

The civilian medical community has benefitted from the military experience in blood banking where significant advances have been made. It should be pointed out that since 1940 much of the research and development in blood grouping, blood banking, and blood transfusion has been, and continues to be, guided by civilian and government committees and institutions. The National Research Council has been foremost in this respect.

SPECIAL PROBLEMS (22-26)

Emphasis will be placed in this section on the discussion of laboratory procedures for the detection of evidence of incompatibility. It is evident that the responsibility inherent in any blood program is the prevention of injury to the recipient from blood transfusion.

In reviewing special problems, it will be noted that there is an unique relationship of factors compromising the achievement of partial or full serologic compatibility that resides *within*, and *completely outside*, the compatibility testing laboratory. These include:

1. Patient identification.
2. Sample identification.
3. Crossmatch procedures.

4. Use of group-specific blood.
5. Use of group O "universal donor" blood.
6. Patient triage and communication.
7. Training programs.
8. Need for more efficient instrumentation.
9. Transfusion reactions:
 - a. Prevention.
 - b. Detection.
 - c. Management.
10. Frozen blood.
11. Blood components.

The leading problem continues to be the fail-safe, proper, complete, and correct identification of (1) the patient and (2) the patient's blood sample. These two factors play a leading role in success or failure in handling a mass casualty situation. A full understanding must first be attained before assuming responsibility for setting up a compatibility testing laboratory in support of any small- or large-scale emergency blood requirement. It certainly must be appreciated that any blood specimen from a patient, other than the one whose name appears on the tube of blood, could receive the most sophisticated compatibility testing which, although compatible for that patient, would be lethal when transfused into the patient named on the tube, especially if there is an ABO incompatibility. In some cases, the only reason a fatality is avoided is through coincidence; that is, the blood given to the A, B, or AB recipient happened to be group O, low titer A and B isoagglutinins and served in the same capacity as group O universal donor blood. However, such occurrences must be ruled out and controlled by the patient sample identification system instituted.

Let us now examine the reverse situation wherein the blood sample is correctly identified, the compatibility tests are reliably carried out, but the blood is brought to the emergency room and, in the confusion, is given to the wrong patient. There are many reasons for this, but, in the main, they concern patient identification, and, to some extent, the training of the staff.

Crossmatch procedures must blend or "bend" with the scope of the casualty situation and this will be discussed later.

The use of group-specific blood requires that:

1. There is sufficient quantity.
2. The patient is properly identified.
3. The blood sample is accurately labeled.
4. The compatibility testing is adequate and properly carried out.

In the event it is decided to use uncrossmatched type-specific blood, time should be allowed for accurate performance of ABO grouping and Rh typing on the patient.

UNIVERSAL DONOR BLOOD (33,34,53)

It may be argued that the confusion attending more than ten or 20 casualties in a nonpermanent installation probably precludes using group-specific blood except on an elective basis. The transition to type- and group-specific blood can be made as soon as the transfusion can be considered elective rather than emergency. Proper crossmatch procedures will indicate when the transfusion can be made safely. (Sometimes this is not possible for two weeks if five or more units have been given.) Procedures for screening group O bloods for use with A, B, and AB recipients will be discussed later.

PATIENT TRIAGE AND COMMUNICATION (35)

Proper delineation of operational authority within the hospital must be established and should include:

1. Designation of emergency transfusion officers to make clinical decisions on hemotherapy until the surgeon takes over.
2. Emergency procedures clearly defined in writing and distributed by a senior committee comprising laboratory, surgical, nursing, and administrative chiefs.

The emergency room should have ample supplies of albumin, saline, (Ringer's lactate), purified protein derivative, and parenteral administration sets (see Annex N). Documentation should be provided by prenumbered temporary identification tags or wristband system. This identification should be used until the patients, as a group, can be reclassified as nonemergency whether or not the patient is comatose.

The use of group O blood will be based on the transfusion officer's evaluation of urgency. Conversion to type-specific blood will be decided by the pathologist on the basis of compatibility tests in each case. Rule-of-thumb changeover points can be both dangerous and wasteful.

TRAINING PROGRAMS (54)

Training sessions must be held. They should emphasize procedural simplicity and proof that participants *definitely* know their roles. Some hospitals conduct annual "disaster day" programs. These include simulated patients, dry runs, in-depth organization of emergency personnel, etc. However, these are time-consuming and expensive. Subsidies for such programs would have to be made available (36). The Programs of Instruction for three courses in the military are described as examples of various application (Annexes A, B, and C).

NEED FOR MORE EFFICIENT INSTRUMENTATION (51)

This refers to the requirement for automated, hemagglutination systems with sample identification, and readout/print-out capability (22, 37, 51).

PERTINENT TRANSFUSION REACTION LITERATURE

The reader is referred to references 38-49 for a detailed account.

FROZEN BLOOD (17,18)

The use of frozen blood with its longer storage capability must be considered for future use. The problems of better preservatives, rejuvenation, thawing technics, storage, and final container are all under continuing research (Fig. 6).



Fig. 6. Liquid nitrogen tank currently in use at the Fort Knox Low Temperature Blood Preservation Unit.

BLOOD COMPONENTS (32,50,52)

Blood components are included in the scheme because of coagulation problems resulting from multiple transfusions; packed cells are used to prevent circulatory overload in older casualties and other considerations.

Evidence is accumulating that increased hemolytic transfusion reactions (HTR) that lead to incompatible hemolytic blood transfusion disease (IHBTB) result from an attempt to supply group-specific, crossmatched, compatible blood in mass casualty situations thereby overextending the transfusion service's true capability. In effect, by trying to supply the best and safest blood, the goal of preventing injury to the recipients of blood transfusion can be compromised. This enigma is the crux of the problem and relates again to patient and sample identification. A recent poll* indicates that receipt of more than 20-30 casualties, each requiring multiple units of blood, would virtually overtax many of the large medical centers in this country. Keeping these points clearly in mind, let us now direct our attention to the procedures available today to provide blood first in a normal, and later in a mass casualty situation.

TEST PROCEDURES (23-25)

Routine tests.

1. Recipients shall receive ABO and Rh type-specific blood except for reasonable qualifying circumstances.
2. A major crossmatch, the reaction of recipient's serum with donor's red cells, is required by the Standards of the American Association of Blood Banks.
3. The minor crossmatch, which tests the donor's serum with the recipient's red cells is optional if the donor's serum has been adequately tested for expected and unexpected antibodies. The minor crossmatch, if performed, should include the same technics as the major crossmatch.
4. Tests for compatibility shall employ methods which optimally demonstrate both agglutinating and coating antibodies, and shall include antiglobulin (Coombs) tests. Antihuman globulin for the antiglobulin test shall meet DBS Standards.**
5. In addition to the preceding methods, compatibility tests employing albumin, albumin-serum, or protease treatment of red blood cells are advisable.
6. If the result of a test is complement-dependent, noninactivated serum collected within the last 48 hours must be used.

*Questionnaire, Annex D.

** Division of Biologics Standards, National Institutes of Health (now, The Bureau of Biologics).

Applicable techniques include:

1. Saline or serum test.
 - a. Room temperature (18-22 C).
 - b. 37 C.
2. High protein test at 37 C.
3. Antiglobulin test.
4. Enzyme test.

STANDARDS FOR EMERGENCY SITUATIONS (25)

There are instances in which delay in blood transfusion may jeopardize life. The following standards apply:

1. Recipients whose ABO and Rh type have been determined by the transfusing facility without reliance upon previous records may receive type-specific blood before tests for compatibility have been completed.
2. Recipients whose ABO type is not known may receive uncross-matched type O blood which has been processed as follows:
 - a. The removal of at least 70% of the plasma from whole blood is the preferred method for reducing the levels of anti-A and anti-B.
 - b. When type O whole blood is to be used for other than type O recipients, it must be free from hemolytic anti-A and anti-B. The label should indicate the result of the test.
3. When type A or B blood is used for type AB recipients, the plasma should be removed or must be free of hemolytic antibodies.
4. The records shall indicate the urgent nature of the situation and shall contain a statement of the attending physician accepting responsibility for omission of compatibility tests.
5. Standard compatibility tests should be completed promptly.

FUTURE GOALS (PLANNING) (Fig. 7)

When we start thinking about any large blood program, certain facts stand out as basic requirements while others become nice to have, but are not critical.



*"But oh, beamish nephew, beware of
the day"*

Fig. 7.* Planning for future periods of exigency is
always plagued by the forecasts of doomsday prophets
that all plans will be a hopeless failure.

Figures 8-11 show a composite of critical requirements.

* Permission to reproduce this figure was kindly granted by Bramhall House. (Carroll, Lewis. The Annotated Snark from The Hunting of the Snark. New York: Bramhall House, 1961, p. 57.)

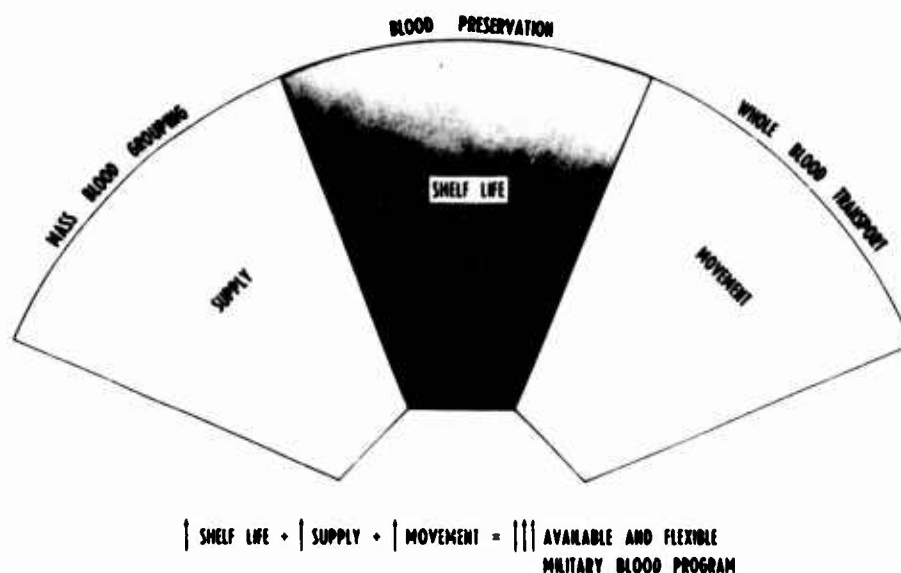


Fig. 8. The spectrum of planning for improving future operations covers a multitude of considerations. Key areas are included in this figure.

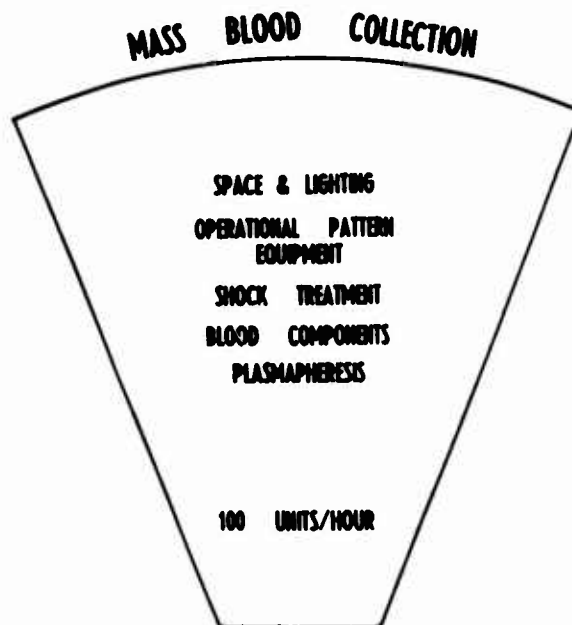


Fig. 9. There are many areas here that require alternate "fail-safe" planning.

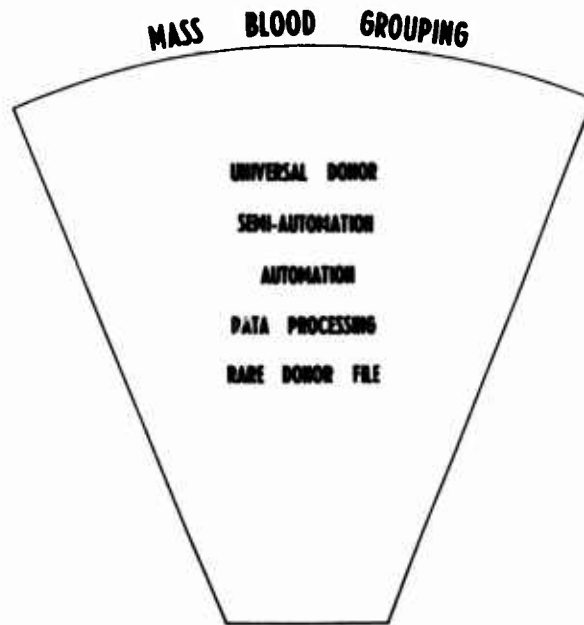


Fig. 10. The greatest refinement will come with sample identification, automation of blood grouping, and a read-out/print-out to include labeling and inventories.

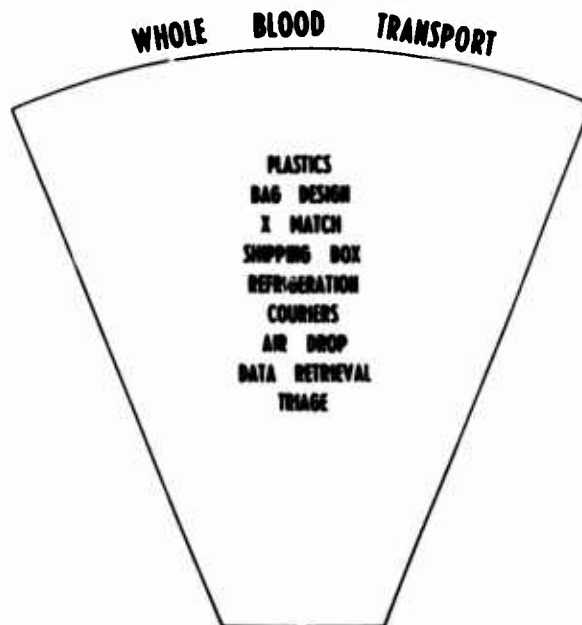


Fig. 11. In addition to this myriad of considerations, we should include cargo coding.

Figure 12 provides a closer look at mass blood collections. Blood donors must be obtained, transported (possibly), brought to a central



Fig. 12. The availability of transported volunteers for mass blood collection is a keystone in planning.

facility, processed (physical exam), bled, and released following recovery and refreshments. The space, lighting, equipment, and *staff to bleed* 100 donors per hour are shown in Figures 13-18.



Fig. 13. Alternate locations for large-scale collections should be located and used on trial runs. Prelocation of equipment should also be considered. Thirty thousand sq. ft. of space are available in this building.



Fig. 14. The space can be used to funnel traffic into different areas of processing, such that 100 donors per hour can be handled on a sustained 10-hour basis.



Fig. 15. Briefings and donor card preparation are conducted in areas adjacent to the bleeding area.



Fig. 16. Once the pipeline is filled, each area becomes a mass turnover of donors.



Fig. 17. Numerous beds and a large, well-trained staff must be at hand.



Fig. 18. Supervisors, trained in managing a large bleeding operation, must be available.

Unless a unit is normally mobile in its collection operations, pre-location of equipment is necessary. Figure 10 relates to factors of rapidly and accurately performing the necessary blood group serology tests as described under emergency procedures. Decisions of drawing only group O, or O and A, or all groups must be made. Automation can be very helpful in this area and represents an answer to fatigue, errors, and requirement for a large staff of trained personnel. What if there is no power? What if there are no blood grouping reagents?

LTC John E. Rogers, Jr., MSC (56), ran into a problem similar or even worse than this during World War II. Read the manner in which he resolved some problems in Annex G.

Figure 11 brings together numerous facets that have critical impact on a successful program. The plastic bag must be available in the collection center and be able to withstand considerable handling. Shipping boxes, wet ice, and continuous refrigeration are problems listed under our transport category. Moving personnel, casualties, and blood is accomplished in a particular region by helicopter (Figs. 19 and 20). It is not always this simple (Figs. 21 and 22).



Fig. 19.* This is a gunship evacuating combat troops from an area. In CONUS, availability of such transport, in certain areas, could be useful in a civil disaster situation.

*Courtesy of US Army Aviation Digest. (Cover, US Army Aviation Digest, March 1971.)



Fig. 20.* The ideal transport of wounded and medical supplies (blood and components) is by way of medical evacuation helicopters.

* Courtesy of Military Medicine. (Cover, Military Medicine, Volume 136, No. 1, January 1971.)



Fig. 21.*



Fig. 22.*

* See footnote on next page.

WE STAND ON THE SHOULDERS OF GIANTS

This monograph describes, in some detail, pertinent areas of military blood banking, unique to our life span, of unusual activity and accomplishment. The contributions of MG Douglas B. Kendrick, BG Robert M. Hardaway, III, and others during World War II and the Korean War are the basis of much of this manuscript. The role of COL William H. Crosby during the Korean program, and later, is also much in evidence. The impact of their tremendous efforts and guidance continues to influence, to a great degree, all major decisions in military blood banking as we know it today.

SUMMARY

Military blood bank experiences are reviewed, especially the highlights encountered in the period 1941-1971 during the World War II, Korean, and Vietnam campaigns. Since these military exigencies served as the impetus for research and development in all facets of blood banking, it was considered a worthwhile task to reexamine the problems, the approaches to resolve the problems, and the various levels of achievement. Many of the large-scale military blood bank operations are directly applicable to civilian blood banking and civil disaster plans and operations. Areas reviewed include collection, processing, shipping, and storage of blood, the group O universal donor, transfusion reactions, hepatitis, blood preservation, blood components, and frozen blood. An interesting finding in Vietnam statistics correlates well with COL William H. Crosby's observations and recommendations in Korea concerning group O universal donor blood versus group specific, crossmatched blood. There were no reported deaths in Vietnam in over 100,000 group O universal donor transfusions; however, group specific, crossmatched blood given during mass casualty situations resulted in 49 reported cases of hemolytic transfusion reactions and nine deaths. Mannitol and hemodialysis were effective in salvaging the other cases of HTR. Based on this experience in two large-scale operations in the military, group O universal donor blood should continue to be considered by medical centers in the United States suddenly faced with numerous casualties in their emergency receiving rooms.

Figures 21 and 22.* Heroism of combat medics. "Those combat medics on bouncing jeeps--kneeling and balancing and clinging miraculously with one arm, raised the other high, as one would a torch, holding a bottle of plasma, pouring life back into a broken body. I think I have never seen a soldier kneeling thus who was not in some way shrouded with a godlike grace and who did not seem sculptured and destined for immortality." [See reference (2).]

* Permission to reproduce these figures was kindly granted by Harper and Bros. (Mydans, Carl. More than Meets the Eye. New York: Harper and Bros., 1959.)

Transfusion reactions, other than hemolytic, and not related to ABO red cell antigen and serum antibodies, occurred in large numbers during the three conflict operations described in this monograph. *It is concluded that the present form of resuscitation, at the point of injury, using group specific whole blood, universal donor blood, plasma expanders, and especially Ringer's solution with lactate (pH 8.5), might well be "augmented" in the near future with blood components, frozen red cells, and rejuvenated red blood cells.* The frozen red cells and rejuvenated red

RINGER'S LACTATE

Ringer's, Injected, Lactated, USP 1,000 6's (unit)
Federal Stock Number (FSN) 6505-299-8615
(Planning guideline: 17.6559 units/1000 men/30 days)

Issued for Vietnam (units of 6's)

Jul 68-Jun 69	90,173
1969	61,470
1970	124,064
1971	140,965
1972	57,527

cells would also serve as a "fail-safe" supply of blood during periods of acute shortage of whole blood. It is recommended that larger treatment centers establish transfusion services that could *routinely* handle transfusion reactions, in addition to hemolytic reactions, which occur in young males following multiple transfusions. Modification of this plan would also handle all male and female casualties in civil disasters. These measures* include the use of: packed red cells, washed red cells, buffy coat-poor and platelet-poor red cells. It also includes the consideration of a frozen red cell bank for rare groups, uncommon groups (A₂B), and as a general backup for any blood shortage and emergency.

The activation, including staff study and growth of the Blood Transfusion Division and The Blood Bank Center of the US Army Medical Research Laboratory, Fort Knox, Kentucky, is used as an example of planning for large-scale blood banking in the military (operations, training, and research) and, consequently, civil disaster blood bank planning. Large-scale blood bank operations in being at Fort Knox are illustrated and described. The growth and rapid expansion at Fort Knox paralleled the Vietnam campaign in both timing and activity. The contributions of MG Douglas B. Kendrick, BG Robert M. Hardaway, III, and COL William H. Crosby are discussed throughout the monograph.

*Includes, especially, the group O red cell antigen combination for low temperature preservation suggested by Dr. Morten Grove-Rasmussen and Dr. Charles Huggins.

Finally, specific areas requiring continued research and development are listed for military blood banking, most of which have application to civilian blood banking.

CONCLUSIONS

Modus operandi during mass casualty situations. It is difficult to lay down directives applicable to all facilities, in all situations, concerning the use of blood or Ringer's lactate.

Implementation of any program to handle mass casualties remains the responsibility of the medical officer charged with such problems. His limitations are: the staff size; the staff expertise; physical plant; and resources on hand, including blood, blood components, and Ringer's lactate.

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SECTION II

US Army Medical Research and Development Command

Monitors of Research Programs

US Army Medical Research Laboratory

Fort Knox, Kentucky 40121

1965 to present

Director, Surgical Research Directorate

COL D. W. Pratt, MC (M.D.)

Chiefs, Surgical Research Division

COL J. J. Kovaric, MC (M.D.)

COL L. R. Rose, MC (M.D.)

COL W. L. Scheetz, MC (M.D.)

COL H. E. Bivens, MC (M.D.)

LTC F. H. Taft, Jr., MC (M.D.)

Chiefs, Blood Transfusion Research Branch

MAJ G. S. Cowan, MC (M.D.)

MAJ J. D. Alford, MC (M.D.)

MAJ B. W. Brient, MC (M.D.)

NEWS RELEASE - OFFICE OF THE SURGEON GENERAL, US ARMY
18 June 1965

TRANSFUSION RESEARCH DIVISION MOVED TO
FORT KNOX MEDICAL RESEARCH LABORATORY

The US Army Medical Research and Development Command's programs for research in blood transfusion and the training of Blood Bank Fellows will be transferred from the Walter Reed Army Institute of Research to the Army Medical Research Laboratory, Fort Knox, Kentucky, effective 1 July 1965.

The Transfusion Research Division, Fort Knox, will undertake the following:

- . Develop and establish a standard reliable system for determining and recording the blood group of all military personnel. The system will employ automation with special emphasis on accurately identifying the "universal donor."
- . Train officers and enlisted men in all phases of military blood banking.
- . Evaluate and develop more reliable reagents, equipment and methods for the collection, processing, preservation and transfusion of blood.
- . Provide consultation service in blood grouping, crossmatching and transfusion problems.
- . Provide a central activity for the study of unusual blood groups and types and the procurement testing of blood grouping and typing reagents.
- . Maintain a rare blood group donor file and a stock of blood components.
- . Conduct research on the indications for blood transfusion and the prevention of injury by transfusion.

The initial staffing of the Transfusion Research Division will include: two officers, 11 enlisted personnel and 17 civilian specialists in blood banking and immunohematology.

The Transfusion Research Division will maintain close liaison with all Government and civilian agencies and institutions engaged in various aspects of blood transfusion, as well as with similar research activities throughout the world.

A Registry of Rare Manuscripts, with English translation, describing early research in the field of immunohematology, blood banking and blood transfusion will be maintained.

MILITARY BLOOD BANKING



The parts (operations, training, and research) effectively contribute to the successful function of the whole (military blood banking) only when they are in the proper spatial relationship to each other. Equidistant in this case does not mean 2,000 miles, 1,000 miles, or even 50 miles; it means a combined effort of these critical parts under one roof such as the Fort Knox laboratories described in this monograph.

THEN AND NOW - A WHIRLWIND DECADE

by

Frank R. Ellis, M.D.



It is ten years since the first serious discussions began in a small, somewhat disheveled laboratory in the Walter Reed Army Institute of Research laboratory in Washington. An outsider-Army Reservist-guest of the Institute served as the Devil's Advocate in a series of rap sessions in 1963-64 during several tours of active duty for training. The thesis developed that during the preceeding 15 to 20 years the Regular military establishment had lost a superb scientific capability in technical blood banking capacity. The apogee of this ability had probably coincided with the great exodus of World War II scientists who resumed their civilian endeavors in 1945-46. In the ensuing 18 years, even the stragglers had been whittled away by retirement, death, or discharge from the service.

We had been pretty much through Korea. Vietnam was on the horizon. The consensus emerged that none of the three Armed Services commanded any genuine leadership in blood banking. Even though the military hospital population was to increase, the formal ability to deal with transfusion related problems seemed destined to respond to the pressures of need just as they had a generation before. The prospects were bleak. Everyone conceded that commercial sources would no doubt continue to be asked to resolve patient problems encountered in military hospitals. Reagents would continue to be purchased with little likelihood of meaningful evaluation prior to finalization of contracts covering large sums of money.

But the dreams and hopes from these discussions took meaningful shape in the 1964 Staff Study of Crosby and Camp. In accordance with the recommendation of this Staff Study, the Blood Transfusion Research Division became an operating part of the US Army Medical Research Laboratory on 1 July 1965 at Fort Knox, Kentucky. A group of distinguished

scientists have served as advisors to the Blood Transfusion Research Division, and its accomplishments are truly legion. Although Army sponsored, it has served as a tri-service institution. It has remained under the imaginative and skilled direction of its founding director, Colonel Frank R. Camp, Jr., and it has achieved in its first decade of operation a commanding position as a dominant scientific force in its field. The highlights of its development begin with the opening of the Blood Group Reference Laboratory in 1965, and the formal monitoring of biologics purchased by the Defense Personnel Support Center in the same year. The training of Blood Bank Fellows has been an important contribution to all three military services. One concern of this reviewer is that so few military physicians have been motivated to go through bench indoctrination when such superb opportunity to do so has been available to them.

Inevitable, it seemed, was recognition by the civilian scientific community of the skills and potential of this laboratory. This recognition began in 1967, with institutional membership and approval for training by the American Association of Blood Banks. This recognition was culminated in 1971 by its designation as an AABB Reference Laboratory. The laboratory has continued to develop through its capacity to provide frozen red blood cells for transfusion, and devising fully automated methods and equipment for the operation of large service blood programs. It has affiliated with academic programs offering graduate degrees in applied immunohematology.

The goals for the immediate future of training noncommissioned officers, special services such as tissue typing, and continuing active planning for military exigencies will keep it a viable and growing institution. The only really troubling thought, one decade from its start, is the unapparent grooming of skilled and dedicated younger officers who must inevitably assume the stewardship of this fine organization. The military mind has failed to appreciate its need with alarming consistency in the past. It must not be permitted to behave so predictably now.

ANALYSIS AND CRITIQUE

by

Alfred Chanutin, Ph.D.



In 1964, COL W. H. Crosby and MAJ F. R. Camp, Jr., stationed at the Walter Reed Army Institute of Research, submitted plans for organizing a Blood Research Division. These plans were initiated to avoid the haphazard, inefficient, and expedient procedures employed during World War II and the Korean War. Fortunately, this program was formulated without anticipating the subsequent blood requirements for combat casualties during the Vietnam Conflict. Fort Knox was chosen as the site for this installation and MAJ F. R. Camp, Jr., was appointed as the first Director of the Blood Transfusion Research Division. Existing temporary buildings were renovated and equipped for the wide variety of tasks to be undertaken and a nucleus of military and civilian personnel was recruited.

A blood bank and ancillary services for blood grouping facilities, packaging, storage, etc., were organized in 1965 according to the Crosby-Camp program. The plan for training military and civilian personnel for operating transfusion services has been highly successful. Research dealing with blood group antibodies and blood grouping has been expedited by automation. Both the staff and physical facilities have been expanded and the many phases of these numerous problems have progressed rapidly and successfully under the present Commander/Director, Colonel Frank R. Camp, Jr.

Another outstanding achievement has been the research done in red cell and platelet metabolism during the storage of blood. Medical officers, chosen for their interest in blood problems, were assigned to duty at Fort Knox. These included:

COL C. E. Shields	1965 to present*
CPT H. F. Bunn	1966-68
CPT L. G. Dauber	1966-68
CPT L. J. Reed	1966-68
CPT H. S. Kaplan	1967-69
MAJ R. B. Dawson, Jr.	1968-71
LTC H. Lopas	1968-71
MAJ N. I. Birndorf	1968-71
MAJ C. E. Bell, Jr.	1968-71
MAJ W. S. Mallin	1971-73
CPT T. R. Poskitt	1971-74
MAJ G. J. Roth	1971-74
CPT J. C. Bobrow	1971-74

The contributions made by these medical officers and by civilian investigators have been fruitful and the well deserved reputation of the US Army Medical Research Laboratory at Fort Knox is a reflection of their efforts. It is worth mentioning a few of the significant projects as follows:

1. The first demonstration that 2,3-diphosphoglycerate decreases the affinity of oxygen for hemoglobin in the *intact* red cell.
2. A significant study of the effects of pH, salt concentration, supplements such as adenine, inosine, and inorganic phosphate on hemoglobin function during blood storage.
3. Nephrotoxic effects of adenine in man and primates.
4. Effect of methylene blue on red cell metabolism during blood storage.
5. A series of monographs which review the literature of blood groups and immunology.

With the advantage of hindsight, it is possible to make the following suggestions:

* One year in Vietnam.

1. Employ a nucleus of competent civilian investigators who could maintain a continuity of interest and experience in a given problem. The assumption that a young medical officer will have the experience, background, and "know-how" to tackle a problem effectively is an exercise in naivete, particularly if his assignment is for a comparatively short time. Of course, there are exceptions!

2. Establish a close relationship between the laboratory and clinical personnel. At times it is essential to test procedures and ideas on man under carefully controlled conditions.

3. Consultants should be called in for advice and suggestions whenever help is needed. If the clinical and laboratory staffs do not have respect for the consultant, he should not be asked to return.

4. Major research problems should be submitted to and discussed with representatives of the US Army Medical Research and Development Command. Once a problem has been approved, the decisions for its implementation should be the responsibility of the Commander or his advisors.

5. There should be limited freedom to pursue fundamental work provided it has some bearing on a practical problem. Large industrial corporations have been following this policy with considerable success.

6. Finally, the successful interaction of the three components at Fort Knox (operations, training, and research) in support of all phases of military blood banking attests quite significantly for the retention of such a concept under one roof.

A CRITIQUE OF PROGRESS AT THE FORT KNOX BLOOD BANK CENTER

by

Paul J. Schmidt, M.D.



It was my privilege to be consulted on the Staff Study relating to the need for a US Army Blood Transfusion Center. The original recommendation "That a US Army Blood Transfusion Service Center be activated and established at Fort Knox, Kentucky, Fort Sam Houston, Texas, or The Presidio of San Francisco, California" meant that we at the National Institutes of Health in Bethesda would lose some good neighbors from the Washington metropolitan area. We had over ten years of "over the fence" cooperation with a real resource, our friends at Walter Reed who were always willing to give us inspiration, encouragement, ideas--and reagents. Even now, we still have some of the original good paper in that was a gift from Joe Akeroyd.

On purely geographic grounds, it was a personal disappointment that Fort Knox was selected. Nevertheless, I was happy that in June of 1965, The Surgeon General of the United States Public Health Service was asked for my continuing services as consultant to the new Transfusion Research Division. The beginnings of a visit to Fort Knox in late February 1967 did little to dispel my original qualms about the site as I was driven on that long murderous road from Louisville in a plains blizzard. However, once I arrived, I saw that the new role of the Army in transfusion research had also arrived. The facilities, the equipment, and most of all, the spirit and caliber of the staff were inspiring. There was much real and needed work under way.

Blood transfusion has always been a very practical field of clinical therapeutics. There is no question that many practical problems were recognized and solved at Fort Knox. The titles of the publications from

the Fort Knox laboratories are a record role of its achievements. Although it was unfortunate that a large hospital transfusion service was not immediately available to the clinicians who had their exposure to blood banking at the laboratory, it was obvious that their training was clinically oriented. Certainly, the publications contained much information directly applicable to hospital hemotherapy.

There has been a continuing cross fertilization between the National Institutes of Health and Fort Knox. Physicians and technologists have gone both ways and several have been permanent members of both staffs in turn.

To look in 1973 at the outcome of the Army's staff planning of 1964 is a gratifying experience. There is no question but that the original simple charge "to conduct research directed to improvement of effectiveness and safety of blood transfusion" has been magnificently met in these past ten years.

A N N E X A
BLOOD BANK FELLOWSHIP

67

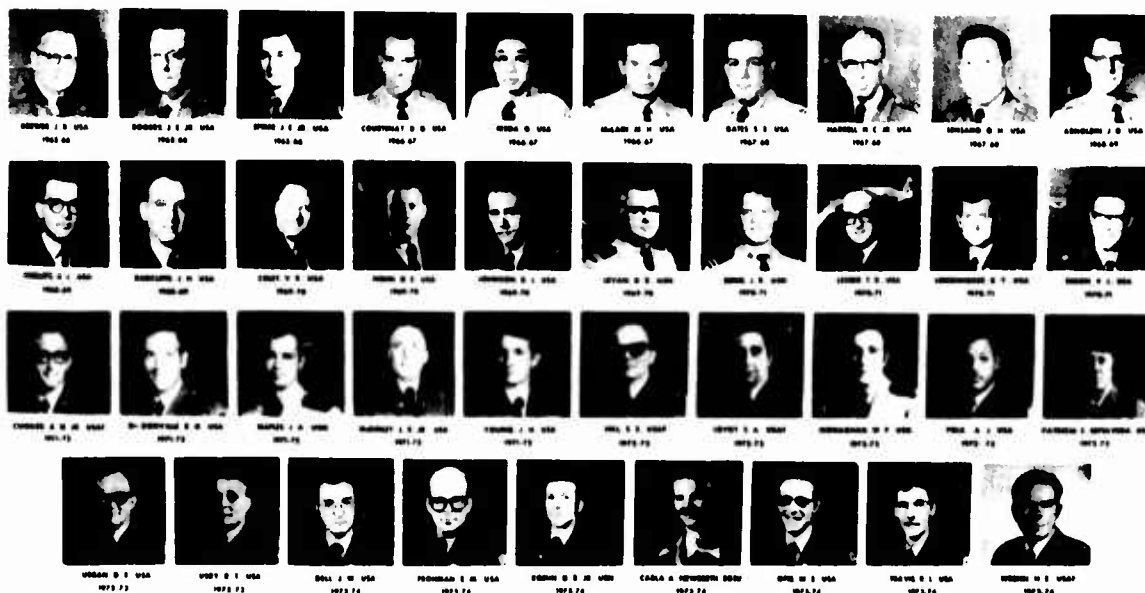
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BLOOD BANK FELLOWSHIP

US ARMY BLOOD BANK FELLOWSHIP PROGRAM WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, D. C. 1958 - 1965



US ARMY MEDICAL RESEARCH LABORATORY FORT KNOX, KENTUCKY



Scope: This course is designed to prepare selected career officers in the laboratory sciences field as blood bank directors of large military hospitals and as coordinators of combat area blood programs. Classroom and laboratory instruction includes all phases of military blood banking, blood grouping (immunohematology), and blood transfusion.

Length: One year.

Location: US Army Medical Research Laboratory, Fort Knox, KY 40121.

Prerequisites: Applicants must be active duty Medical Service Corps officers, with bachelor's degree, in grade of lieutenant or captain; possess or qualify for a laboratory sciences MOS; have a minimum of one year civilian and

one year military, or two years military experience in hospital or medical laboratory. Applications from Navy and Air Force commissioned officers will be considered on an individual basis. Interim TOP SECRET security clearance is necessary.

General Information: Course Sponsor: DASG-PSC-L
Frequency: Annually.

Obligated service incurred by attendance. One year in addition to any other obligated service presently incurred (AR 350-100).

The Blood Bank Fellowship is a 52-week residency program, approved by the American Association of Blood Banks and conducted by The Blood Bank Center, US Army Medical Research Laboratory, Fort Knox, Kentucky. This course provides the training necessary to take the examination for certification as Specialist in Blood Bank Technology, MT(ASCP)BB, or BB(ASCP).

By arrangement with Bowling Green State University, Ohio, Fellows accepted as graduate students may receive up to 45 quarter hours of graduate credit. Part of the credit will apply toward resident requirements for the Master of Science degree and the remainder will apply toward the post-masters degree of Specialist in Applied Biology (Immunohematology). Additional graduate work will allow Fellows to earn either one or both degrees.

The objectives of these programs are to qualify Fellows to operate blood banks, but more important, it will qualify them to implement, in laboratories which are in support of clinical operations, new procedures to utilize the progress in research that is being made in the fields of blood banking, transplantation, and forensic science.



Charles A. Leone, Ph.D.

Dean Graduate School,
Bowling Green State University, Ohio



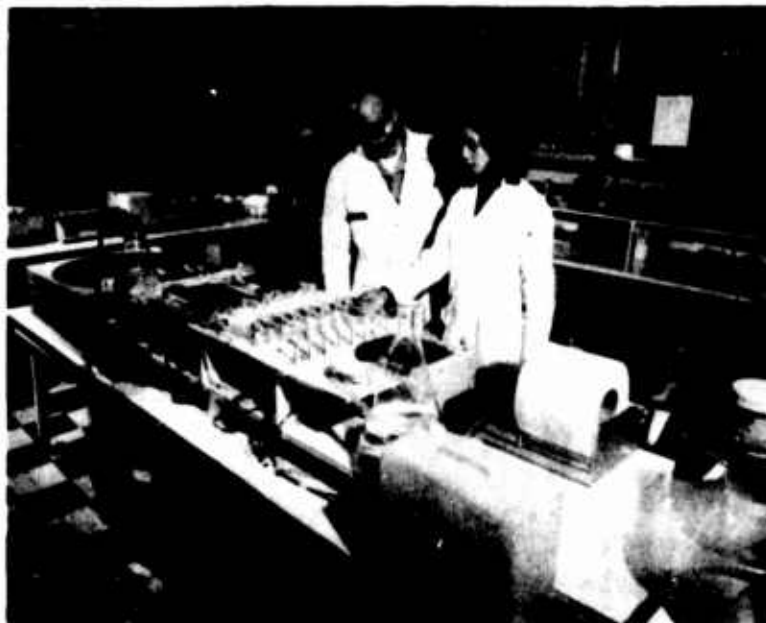
Computer education, Bowling Green State University, Ohio.



Campus scene, Bowling Green State University, Ohio.



Blood group genetics class, USAMRL - an affiliated campus with Bowling Green State University, Ohio.



Instruction in automated blood grouping, USAMRL.

Program of Instruction

Training includes instruction in all phases of the following:

1. Blood Bank Organization and Management:

Medical director and staff
Quarters
Administration
Personnel procedures
Equipment and supplies
Medicolegal problems

2. Blood Donors:

Donor selection
Bleeding procedure
Donor reactions
Processing requirements for donor blood

3. Antigen-Antibody Reactions:

Basic immunology
Factors affecting the *in vitro* demonstration of antigen-antibody reactions

4. The Antiglobulin Reaction (Coombs Test):

Uses of the antiglobulin test
Factors affecting the test
Sources of error

5. The ABO Blood Group System:

Approximate frequencies of ABO phenotypes
Methods for ABO grouping
Subgrouping of A or AB blood
Discrepancies between cell and serum grouping
Sources of error
Heterospecific (ABO) transfusion
Inhibition test for ABH secretor status

6. The Rh-Hr Blood Group System:

Practical considerations in Rh typing
Rh typing
Technics

7. Other Blood Group Systems:

Lewis
Kell
P
Duffy
MNSs

Kidd
Lutheran
Ii
Vel
Additional antibodies

8. Compatibility Testing:

- General procedures
- Technics for compatibility testing
- The incompatible crossmatch
- Exchange transfusions
- Emergency crossmatch
- Changing to group specific blood after transfusion of group O blood
- The group AB recipient
- Massive transfusions
- Crossmatch in presence of prolonged clotting time
- Compatibility testing after infusion of synthetic plasma expanders
- The unit of blood containing an irregular antibody
- Autoimmune hemolytic anemia

9. Blood Group Antibodies:

- Antibody detection
- Antibody identification
- Absorption
- Elution
- Titration of antibodies
- Freezing of red cells for laboratory reagent
- Concentration of antibodies
- Platelet antibodies
- White cell antibodies

10. Hemolytic Disease of the Newborn:

- Antenatal studies
- Neonatal studies
- Selection of blood for exchange transfusion

11. Blood Transfusion:

- Fresh blood
- Small units of blood
- Extracorporeal circulation
- Massive transfusions
- Packed red blood cells (human)
- Transfusions to supply platelets
- Autologous transfusion
- Plasma
- Products derived from human blood
- Complications of transfusion
- Transmission of disease

12. Blood Components:

- Technics of preparation

13. Blood Storage and Transportation:

- Hematological and biochemical characteristics of stored blood

Refrigeration
Donor blood inspection
Sterility (bacteriology) procedures
Requirements for reissue of blood
Anticoagulants
Anticoagulant formulas
Storage of blood in the frozen state
Transportation

14. Labels:

Label requirements and recommendations

15. Records:

Donor records
Supplementary donor records
Record of blood from other banks
Record of blood inspection
Record of refrigeration temperatures
Record of blood culture
Record of laboratory processing
Transfusion request records
Release records
Transfusion records
Records of transfusion complications
Records of distribution or issue
Packed red blood cells (human)
Single donor plasma (human) fresh frozen
Leukocyte-poor whole blood (human)
Leukocyte-poor red blood cells (human)
Platelet-rich plasma (human)
Platelet concentrate (human)
Blood group identification card (wallet)

16. National Clearinghouse Program:

Organization
Details of operation
Fees, statements, and forms
Coordinator-affiliate relationship
Reciprocity with the American National Red Cross

17. Reference Laboratories and Central File for Rare Donors:

Serologic problems
Reference laboratory request form
Central file for rare donors
Rare donor registration form
Depot for frozen storage of rare type donor blood

18. Forensic Procedures:

Typing dried blood and serum

Determination of species in pathological specimen
Bone typing for cadaver remains

19. Statistical Procedures:

Experimental design
Statistical methods
Evolution of data
Computer applications

20. Military Blood Banking:

History
Military exigency and field operations
Civil disasters
Other unusual circumstances

General Information

1. At the beginning of the training course, each student will receive a copy of *Technical Methods and Procedures* of the American Association of Blood Banks and a notebook containing the following:

- a. AABB requirements for certification in blood banking.
- b. The Blood Bank Center curriculum and schedule for students in training.
- c. Worksheets for procedures required in each department.
- d. Technical literature:

(1) Standards for a Blood Transfusion Service.

(2) National Institutes of Health Minimum Requirements for Whole Blood (Human); Normal Human Plasma; Single Donor Plasma (Human); Packed Red Blood Cells (Human); Blood Grouping Serum; Anti-Rh Typing Serum; and Antihuman Serum for Antiglobulin Test.

2. Students will receive other texts to study.

3. Lectures on basic sciences (immunology, immunohematology, physiology, genetics, and bacteriology) are scheduled on a regular basis and published monthly.

4. Seminars are conducted by faculty and attended by students and some blood bank staff members. A Journal Club is scheduled weekly, at which time students report on both independent and assigned reading of recent literature.

5. Quizzes are given and notebooks graded monthly.
6. During the last month of the time assigned to the laboratory, each student works independently on investigative problems (transfusion reactions and crossmatch problems) with the technical supervisor available for checking results and answering questions.
7. Library facilities of USAMRL are available to students on a 24-hour basis. Interlibrary loan facilities are available for literature not found at this installation.
8. Throughout the year students spend approximately 12 weeks visiting other blood banks, laboratories, hospitals, and facilities. Subjects covered during these visits are included in the program of instruction.

Curriculum (52 Weeks)

1. Blood Procurement - 9 weeks

a. Medical history (2 wk):

(1) Knowledge of minimum physical standards required for donors and basis of medical history questions.

(2) Practical work in medical tests and in procedures for securing correct information.

b. Phlebotomy technics (7 wk):

(1) Familiarity with all phlebotomy equipment.

(2) Practical experience in correct procedures for drawing blood.

(3) Thorough understanding of national, state, and local requirements for all aspects of blood procurement.

2. Laboratory - 41 weeks:

a. Routine processing and dispensing (14 wk):

(1) Processing laboratory:

(a) Routine ABO groupings.

(b) Rh typing (including testing for D^u).

(c) Serology.

(d) Antibody screening.

(e) HBAg testing:

1. Immunodiffusion.
2. Immunoosmoelectrophoresis.
3. Radioimmunoassay.

(f) Records (medical history cards and registration sheets).

(2) Shipping laboratory:

- (a) Labeling and inspection of processed bloods.
- (b) Storage (refrigeration).
- (c) Shipping (stocking levels, special orders, out-of-town shipments, shipments to other blood banks).

b. Blood component preparation (6 wk):

(1) Preparation of unsuspended packed red blood cells (human); leukocyte-poor packed red blood cells; platelet-rich plasma; platelet concentrate; fresh-frozen plasma; and cryoprecipitate.

- (2) Pooling, storage, and shipping of plasma.
- (3) Culturing.
- (4) Quality control:
 - (a) Red blood cells.
 - (b) Platelet preparation.
 - (c) Cryoprecipitates.
 - (d) Fresh frozen plasma.
 - (e) Fresh frozen plasma (modified).

(5) Records.

c. Coagulation laboratory (1 wk):

- (1) Action of various types of anticoagulants.
- (2) Coagulation factors.

d. Investigative studies (14 wk):

- (1) Crossmatching methods.
- (2) Investigation of transfusion reactions.
- (3) Antibody detection (use of cell panel, enzymes, etc.).

e. Low temperature blood preservation (cryobiology) (4 wk):

- (1) Search for uncommon and rare donors.
- (2) Familiarization of accepted freeze/thaw methods of blood preservation:

- (a) Low glycerol preservation (rapid freeze utilizing liquid nitrogen).

- (b) High glycerol preservation (slow freeze utilizing mechanical freezers).

- (3) Preparation of frozen red blood cells for transfusion:

- (a) Batch wasting.
 - (b) Agglomeration.
 - (c) Continuous flow reduction.

- (4) Cryopreservation of leukocytes and platelets.

f. Experience in blood bank practices (2 wk):

- (1) Observation of storage and record keeping.
- (2) Observation of crossmatch methods and patient/donor identification.
- (3) Observation of administration of blood.
- (4) Blood transfusion reaction procedures.

3. Administration and Medical Records - 2 weeks:

a. Recruitment of donors, with an indoctrination into the various types of donations available; proper approach and attitude; the media available for donor recruitment; publicity.

- b. Scheduling of donors; correlation between blood inventories and special blood requirements, heart surgery, previously scheduled group donors, etc.
- c. Donor registration; the medical history card; assignment of donor and blood numbers; recording statistics.
- d. Identification of donor; value of checking previous donations and medical history with current information.
- e. Review of all regulations--national, state, and local--pertaining to the above procedures.
- f. Purchasing; maintenance of supply inventories; clearinghouse procedures.
- g. Scientific writing.

Lectures

1. Hematology;

- a. Heme biosynthesis, the porphyrias and porphyria.
- b. Iron metabolism and iron-lack anemia.
- c. Globin biosynthesis and sickle cell disease.
- d. Normal red cell production.
- e. Pernicious anemia and the non-Addisonian megaloblastic anemias.
- f. Red cell metabolism and methemoglobinemia.
- g. Red cell destruction and hemolytic disorders.
- h. Polycythemia.
- i. The anemias of bone marrow failure.
- j. Leukemia.
- k. Plasma cell myeloma, primary macroglobulinemias, and other gammopathies.
- l. Lymphomas.
- m. Hemostasis and blood coagulation.

- n. Initiation of coagulation.
- o. Thromboplastogenesis.
- p. Thrombogenesis.
- q. Fibrin formation.
- r. Vascular hemorrhagic disorders.

2. Genetics:

a. The physical basis of heredity:

- (1) The human chromosomes.
- (2) Mitosis.
- (3) Classification of chromosomes.
- (4) Meiosis.
- (5) Human spermatogenesis.
- (6) Human oogenesis.
- (7) Fertilization.

b. Patterns of transmission of genes and traits:

- (1) Autosomal inheritance.
- (2) Sex-linked inheritance.
- (3) Variations in the expression of genes.

c. Chromosomal aberrations:

- (1) Aberrations of chromosome number.
- (2) Aberrations of chromosome structure.
- (3) Chromosomal mosaicism.
- (4) Causes of chromosomal aberrations.
- (5) Clinical aspects of aberrations of the autosomes.

- d. The sex chromosomes:
 - (1) The sex chromosomes and sex chromatin.
 - (2) Intersex.
 - (3) The Lyon hypothesis of gene action on the X chromosome.
- e. Immunogenetics:
 - (1) Transplantation.
 - (2) Autoimmune disease.
- f. Blood groups and serum proteins:
 - (1) Blood groups.
 - (2) Serum proteins.
- g. Population genetics:
 - (1) Systems of mating.
 - (2) Mutation.
 - (3) Selection.
 - (4) Genetic drift.
 - (5) Genetic flow.
 - (6) The origin of races.
- h. Twins in medical genetics:
 - (1) Monozygotic and dizygotic twins.
 - (2) Relative frequency of monozygotic and dizygotic twins.
 - (3) Frequency of twin births and other multiple births.
 - (4) Determination of the zygosity of a twin pair.
 - (5) Limitations of the twin method.
 - (6) Some examples of twin studies in medical genetics.
 - (7) Unusual types of twins.

1. Genetics in medical practice and public health:

- (1) Family history.
- (2) Genetics in clinical practice.
- (3) Genetics in forensic medicine.
- (4) Genetics in counseling and prognosis.
- (5) Eugenics and the relaxation of natural selection.
- (6) Public health aspects of genetics.

3. Serology and Antigenic Systems:

- a. Microbial antigens and toxins.
- b. Tissue and cellular antigens.
- c. Clinical aspects of viral hepatitis.
- d. Detection of hepatitis B antigen.
- e. Characterization of hepatitis B antigen.
- f. Hepatitis B antigen in blood donors.
- g. Posttransfusion hepatitis.

4. Immunology:

- a. Mechanisms of native immunity.
- b. Mechanisms of pathogenicity and virulence.
- c. Mechanisms of acquired immunity--antibody as a specific immune mechanism.
- d. Mechanisms of acquired immunity--the origin and nature of antibody globulin.
- e. Mechanisms of acquired immunity--general characteristics of antibody reactions.
- f. Mechanisms of acquired immunity--the mechanism of antigen-antibody reactions.
- g. Mechanisms of acquired immunity--serologic manifestations of antibody activity.

h. Mechanisms of acquired immunity--the protective effects of antibody.

5. Bacteriology:

- a. Sources of contamination in stored blood and blood components.
- b. Microorganisms encountered in a blood transfusion service, including growth and optimal temperature requirements.
- c. Principles and methods of identification of organisms.
- d. Sterilization technics, use of chemical and physical disinfectants.
- e. Sterility testing as applied to blood banking.
- f. Serologic tests for syphilis.

6. Physiology:

- a. Review of vascular system and blood components.
- b. Blood loss and shock.
- c. Blood survival *in vitro* and *in vivo*.
- d. Storage of whole blood, packed cells, washed cells, pooled and frozen plasma.
- e. Low temperature blood preservation.
- f. Low temperature platelet and leukocyte preservation.

7. Applied Immunology:

- a. ABO blood groups.
- b. Rh blood group system.
- c. MNSs blood groups.
- d. P blood groups.
- e. Lutheran blood groups.
- f. Kell blood groups.
- g. Lewis blood groups.

- h. Duffy blood groups.
- i. Kidd blood groups.
- j. Diego, Cartwright, Dombrock, and Auberger blood groups.
- k. I blood groups.
- l. Frequent antigens.
- m. Infrequent antigens.
- n. Problems in parentage, identity, and blood groups.
- o. Twinning, chimerism, and dispermy.
- p. Mapping of the autosomes.
- q. Xg, the X-chromosome and aneuploidy.

8. Additional Didactic Training:

- a. Blood donation and the transfusion of red cells.
- b. Transfusion of platelets, leukocytes, and plasma components.
- c. Blood volume.
- d. Transfusion in oligemia.
- e. Leukocyte, platelet, and serum antigens.
- f. Autoantibodies.
- g. Incompatibility: shortening of posttransfusion survival.
- h. Hemolytic transfusion reactions.
- i. Other unfavorable effects of transfusion.
- j. Hemolytic disease of the newborn.

9. Preparation of Blood Components:

- a. Use and calibration of the centrifuge.
- b. Preparation of:
 - (1) Packed red blood cells.

- (2) Fresh frozen plasma.
- (3) Platelet-rich plasma.
- (4) Platelet concentrate.
- (5) Leukocyte-poor red blood cells.
- (6) Single donor plasma.
- c. Indications for blood components.
- d. Administration of components.
- e. Regulations regarding shelf life, labeling, NIH requirements.
- f. Sterility controls.

Seminars, Discussion Groups, and Graduate Courses

1. Journal Club meeting weekly to review current literature of blood bank significance.
2. Seminars and Discussion Groups:
 - a. Anticoagulants and blood preservation.
 - b. Psychology of donor and patient.
 - c. Blood group systems other than ABO and Rh (gene frequencies, population formulas, ethnic incidence, antigenicity, clinical significance, laboratory methods).
 - d. The blood bank as part of the hospital organization.
 - e. Medicolegal aspects.
 - f. Blood donor selection, processing, reactions.
 - g. Forensic applications of tissue and bone typing.
3. BGSU Graduate Course Topics:
 - a. Immunoassay.
 - b. Immunohematology.
 - c. Biostatistics.

- d. Physiology of circulatory system.
- e. Supervised blood banking practices.
- f. Operations and management of blood banks.
- g. Field service.
- h. Biological documentation techniques.

Training Visits During Field Service

1. American Red Cross:

- a. Louisville, Kentucky.
- b. National Headquarters, Washington, D. C.--Dr. Tibor J. Greenwalt.
- c. Regional Headquarters, Washington, D. C.--Dr. Evan C. Stone.
- d. Red Cross Blood Center, Boston, Massachusetts--Dr. Allan Kliman.
- e. Red Cross Blood Center, Toledo, Ohio.

2. National Institutes of Health, Bethesda, Maryland:

- a. Bureau of Biologics, Food and Drug Administration--Dr. Sam T. Gibson.
- b. Clinical Center Blood Bank--Dr. Paul Schmidt.

3. United States Navy:

- a. Navy Blood Research Laboratory, Chelsea Naval Hospital, Chelsea, Massachusetts--Cdr C. Robert Valeri.
- b. National Naval Medical Center, Bethesda, Maryland--Chief, Pathology Service.

4. United States Army:

- a. Walter Reed General Hospital, Washington, D. C.--Chief, Pathology Service.
- b. Walson Army Hospital, Fort Dix, New Jersey--Chief, Pathology Service.
- c. Brooke General Hospital, Fort Sam Houston, Texas--Chief, Pathology Service.

d. Ireland Army Hospital, Fort Knox, Kentucky--Chief, Pathology Service.

5. Other Federal Agencies:

a. Federal Bureau of Investigation, Washington, D.C.--Dr. Briggs White.

b. Armed Services Whole Blood Processing Laboratory, Fort Dix, New Jersey.

c. Military Blood Program Agency, Washington, D. C.

d. Defense Medical Materiel Board, Washington, D. C.

6. Commercial Laboratories:

a. Ortho Diagnostics Laboratories, Raritan, New Jersey--Dr. Philip Levine.

b. Pfizer (Knickerbocker) Laboratories, New York, New York.

c. Hyland Laboratories, Los Angeles, California.

d. Dade Reagents, Inc., Miami, Florida.

e. Chicago Blood Donor Service.

7. Civilian Laboratories:

a. Michael Reese Research Foundation, Chicago, Illinois--Dr. Aaron Josephson.

b. Boston Blood Grouping Laboratory, Boston, Massachusetts--Dr. Irving Umansky.

c. Cytology Laboratory of Blood Research Institute, Inc., Boston, Massachusetts--Dr. James L. Tullis.

d. Laboratories of Dr. A. S. Wiener, New York, New York.

e. New York Blood Donor Center, New York, New York--Dr. Fred H. Allen, Jr.

8. Civilian Hospitals:

a. Massachusetts General Hospital, Boston--Dr. Charles Huggins.

b. Children's Hospital, Boston, Massachusetts--Dr. Sherwin V. Kevy.

c. Mt. Sinai Hospital, New York, New York--Dr. Richard E. Rosenfield.

9. Defense Personnel Support Center Workshops:

a. The concept of a "total blood banker and immunohematologist" is offered to the Fellows of this program when they make their training visits with the many experts in this field. As is often the case, the military has relied on experts in the civilian community as consultants in the technical fields. The visits of these consultants in their laboratories offer the Fellows knowledge of the arts and sciences of this new medical discipline as it is practiced in this country. This type of training enriches the program and maintains an advanced state of practice in the blood banks and transfusion services by graduates of the Fellowship.

b. As the duties in this field become more diverse and specialized within the Armed Services, the Blood Bank Fellowship Program offers the training to selected individuals in fulfillment of the requirement for highly trained personnel in peace as well as in times of crisis.

c. The instructors and Fellows of this program form a staff of highly qualified personnel for routine or special consultation services in the field of blood banking and immunohematology for the improvement of the entire Armed Forces Blood Program. This consultation service is available to all military units on request.

d. Through the present tri-service concept, the Armed Services have within their structure a unique and outstanding training program.

A N N E X B

**TRANSFUSION OFFICER TRAINING PROGRAM
FOR MEDICAL CORPS OFFICERS**

**TRANSFUSION OFFICER TRAINING PROGRAM
FOR MEDICAL CORPS OFFICERS**

An intensive six-week course is offered to qualified Medical Corps candidates at the US Army Medical Research Laboratory, Fort Knox, Kentucky. The quota of students is limited to two per course. Four courses per calendar year are offered.

The Transfusion Officer Training Program provides didactic instruction, laboratory practice, and field experience in the following areas:

- a. Blood collection, processing, storage, and shipment.
- b. Blood grouping, blood banking, and blood transfusion.
 - (1) Manual.
 - (2) Semiautomation.
 - (3) Full automation.
- c. Advanced immunohematology.
- d. Special problems including forensic aspects.
- e. Blood preservation research and related areas.
- f. Plasma salvage programs.
- g. Blood component therapy.
- h. Plasma expanders.
- i. Training programs, workshops.
- j. History of military blood banking.
- k. Triage of blood - problem areas.
- l. Pitfalls of blood grouping, blood banking, and blood transfusion.
- m. Shock - current concepts.
- n. Administration in the Continental United States; Department of Defense Military Blood Program Agency; American Red Cross; American Association of Blood Banks.

- o. Transportation and equipment.
- p. Transfusion equipment.
- q. Quality control of blood grouping reagents and equipment.
- r. Human errors in military blood banking.
- s. Communication - the feedback mechanism.
- t. Assigned projects and reports.
- u. Role of the theater transfusion officer.
- v. Liaison with three services and other agencies.
- w. Government agencies, industry, and university contacts.

A N N E X C
PROGRAM OF INSTRUCTION FOR COURSE 311-F1

PROGRAM OF INSTRUCTION
FOR
COURSE 311-F1

Blood Grouping, Collecting, and Processing

Length: Six Weeks

311-F1 Instructor and Students:





Purpose: To provide enlisted personnel with a working knowledge of the principles and technics of collecting, grouping, and processing whole blood in a large military blood bank center on a routine or emergency basis.

<u>A</u>	<u>B</u>
<u>Nonacademic Hours</u>	<u>Academic Subjects</u>
In processing 4 Out processing 4 Commander's time 6 Graduation exercise 2 Class photo 1 17 hr	Orientation 1 Basis of blood banking 4 ABO system 2 Rh system and other blood groups 2 Antibody detection 6 Blood Donor Center 6 Blood processing 32 Daily operations 94 Special topics 11 Written exams 7 Practical exams 58 223 hr
<u>C</u>	
<u>Recapitulation</u>	
Practical exercises 122 Lectures 36 Examinations 65 Nonacademic 17 240 hr	

A N N E X D
QUESTIONNAIRE

70

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QUESTIONNAIRE

1. One problem of modern blood banking is the scarcity of approved guidelines in handling the mode of blood therapy in a semi-mass casualty situation that presents itself initially with the arrival of 50-100 casualties in the emergency room.

2. To define the scene further, we can visualize an emergency room of a large general or city hospital receiving patients in these numbers at 10:30 A.M. when the full hospital staff is available, and normally engaged in routine and emergency patient care. Secondly, let us consider the same type of situation occurring at 2:30 A.M. when a skeleton staff is available. Questions that come to mind are these:

- a. Mode of patient identification.
- b. Extent of use of group O universal donor blood. How long?
- c. Transition to group-specific blood.
- d. Crossmatch procedures.
 - (1) Donor sample - how identified?
 - (2) Oxalate or clot.
 - (3) Technique: saline, high protein, Coombs test.
 - (4) Dispatch and/or pickup of blood.
- e. Firm liaison and continuing aspects of sorting between surgical staff and pathologist.
 - (1) Recommendations for effecting this; e.g., it may be part of the original patient identification procedure or an adjunct to it.
 - (2) The role of training program to achieve some of these goals - dry runs, seminars, etc., of surgical, emergency room, and laboratory staffs, including immediate training of replacements in these various services.

A N N E X E

**STAFF STUDY
"US ARMY BLOOD TRANSFUSION SERVICE CENTER"**

STAFF STUDY

"US ARMY BLOOD TRANSFUSION SERVICE CENTER"

Prepared at Walter Reed Army Institute of Research
Washington, D. C. (1964)

by

W. H. Crosby and F. R. Camp, Jr.

Subject: US Army Blood Transfusion Service Center (Incl 2)

1. Problem. To determine whether there is a requirement for the establishment of a US Army Blood Transfusion Service Center.

2. Assumption. It is unlikely that technical or methodologic improvements in patient care or resuscitative fluids will, in the foreseeable future, result in any major change in the requirement for whole blood or the component parts thereof. The current Army Medical Service Mobilization Plan (U), 11 Nov 63, Appendix 13 - Whole Blood Plan, will remain in effect.

3. Facts Bearing on the Problem.

a. The military requirement for a blood transfusion service is set forth in the Army Medical Service Mobilization Plan (U), 11 Nov 63, Appendix 13 - Whole Blood Plan. While this plan is sound, the means for implementing the plan and a facility for meeting emergency requirements do not exist.

b. The present number of trained transfusion service personnel would not enable a rapid expansion of this service.

c. There is no adequately supported in-service facility with a mission:

(1) To develop and test transfusion equipment and improved methods for preservation and shipment of blood.

(2) To survey, on a continuous basis, the logistical aspects of the transfusion service with a view to improvement and reduction of cost.

(3) To maintain a systematic surveillance of equipment and methods.

(4) To investigate clinical problems in blood transfusion and other immunohematological problems.

(5) To conduct research directed to improvement of effectiveness and safety of blood transfusion (pathophysiology of adverse effects of transfusion).

(6) To define parameters of successful transfusion.

4. Discussion.

a. In 1939, at the outbreak of World War II, the United States found itself with no organized blood bank system and, in fact, had no plans for supplying whole blood or blood substitutes within the Theaters of Operation. By 1941, when this country entered the war, the plasma program was beginning to evolve, but a whole blood program had not reached the planning stage. Both programs developed by a series of expedients, almost on a trial and error basis, and although successful, the success was achieved at the cost of delay, inefficiency, and far greater expense than should have been incurred. Moreover, the tremendous opportunity for research afforded by the collection of millions of units of blood and its clinical use in war casualties, partly as whole blood and partly in the form of plasma and serum albumin, was, for the most part, lost.

b. When the Korean War broke out in June 1950, less than 10 years after the United States had entered World War II and just 5 years after World War II had ended, planning for a blood bank system had been instituted, but so shortly before the beginning of hostilities that, as in World War II, planning and implementation again were carried out on a basis of expediency.

c. In the Fall of 1962, a call for whole blood for military exigency resulted in some disappointing experiences. The medical installations, upon which the whole blood requirement was levied, complained even though the amount of blood requested was not excessive. Once the blood was obtained there was no clear-cut plan for shipping it. Finally, when the blood arrived at its initial destination some of the units were found to be unsatisfactory because the integral donor tubing had been improperly sealed. This was the result of untrained personnel doing the bleeding and lack of trained supervisory personnel.

d. An emergency today would find the Blood Transfusion Service unprepared and with no detailed and coherent plan for implementing the Whole Blood Plan of the Army Medical Service Mobilization Plan.

e. The state of readiness implicit in the Mobilization Plan would be greatly improved by the establishment of an Army Blood Transfusion Service Center adequately staffed and located in an area of troop concentration to provide an adequate blood donor population, and with adequate communications to permit economical shipment of blood by air or rail. The Army Blood Transfusion Center would be comprised of:

(1) A Blood Donor Center on continuous alert and in continuous operation providing blood for the Transfusion Services of the Army's continental hospitals, but which could be immediately diverted to meet any emergency requirement. Collection, processing, and shipping would be carried out at all times as though for an emergency requirement except the destination of the blood would be to Army hospitals in the ZI and occasionally overseas to test the system. In this manner the emergency procedure would remain in continuous use and under continuous study for improvement (see Incls 1-4).

(2) A unit from which cadres could be detached rapidly to establish satellite Blood Donor Centers at other designated areas of troop concentration. These bleeding and processing teams would be immediately available for surface or air travel to any area (see Incls 1 and 2).

(3) A training center for personnel for the operation of the Army's transfusion services. This would include:

(a) Blood Bank Fellowship training for officers and for military and civilian technicians.

(b) Training of specialty teams (TOE 8-500, cells NA and NB).

(c) Blood bank workshops and seminars (see Incl 1, para 3-7).

(4) A laboratory for the development and testing of transfusion equipment and improved methods for preservation and shipment of blood. Studies which could begin immediately include:

(a) Increasing the shelf life of whole blood from 21-42 days by the addition of adenine to the standard ACD solution.

(b) Air drop of whole blood by various means.

(c) A study of the problem of centrifugal speeds and development of a more versatile centrifuge for use in blood grouping.

(5) A staff for continuous surveillance of the logistical aspects of the transfusion service so that any development which can improve the effectiveness or reduce the cost of the transfusion service could be promptly incorporated into the Mobilization Plan. The aspect of automation in blood grouping, blood typing, and records processing is indicated here in the form of the AutoAnalyzer for mass blood grouping coupled with a data processing capability. Several significant needs of the service can be cited here:

(a) Highly accurate results obtained from blood grouping of recruits and blood donors.

(b) Highly accurate records (dog tags, donor cards, and blood unit labels).

(c) Elimination of re-testing by a central collection unit.

(6) A reference laboratory for clinical problems in blood transfusion and other immunohematologic problems. The reference laboratory would conduct research and provide service in the following areas:

(a) Identification of blood group antibodies.

(b) Complete blood grouping facilities.

(c) Storage of rare cell panels in liquid nitrogen.

(d) Consultation in blood transfusion problems.

(e) Forensic studies on blood stains, saliva, and semen.

(f) Genetic studies.

(g) Control studies on standard antisera and transfusion equipment.

(h) Conduct immunohematology workshops.

(i) Leukocyte and platelet antibody studies.

(j) Hepatitis studies.

(k) Data processing system.

(l) Application of automation to blood grouping and blood banking. (See Incl 1, para 3-7; Incl 4, para 1-5.)

(7) A research laboratory with a mission directed to improvement of effectiveness and safety of blood transfusion. Specific studies, projects, or problem areas would be designated by the Research and Development Command.

(8) A Blood Transfusion Service Center Administrative Headquarters.

5. Conclusion. The US Army Blood Transfusion Service Center should be established as rapidly as possible to provide a facility for implementing the Military Blood Mobilization Plan and to provide training and technical support to US Army medical installations.

6. Action Recommended.

a. That a US Army Blood Transfusion Service Center be activated and established at Fort Knox, Kentucky, Fort Sam Houston, Texas, or The Presidio of San Francisco, California.

b. That the Center be activated immediately with a staff of ten and with a plan to enlarge its size and capability over a period of two years until it achieves the ultimate size and optimum capability to carry out its assigned mission (see Incl 5).

c. That the mission be assigned as outlined in paras 3c and 4e, above.

Additional Considerations Relating to the Capability
of the Blood Transfusion Service Center

1. In addition to collecting, processing, and shipping of whole blood, the US Army Blood Transfusion Service Center would also:
 - a. Collect, process, store, and distribute blood components.
 - b. Perform plasmapheresis for rare and convalescent plasma.
 - c. Conduct laboratory and field studies on techniques, reagents, equipment and problems related to all aspects of Military Blood Banking.
 - d. Maintain rare donor and hepatitis files.
 - e. Maintain data processing systems.
2. Each cadre for emergency activation of satellite Blood Donor Centers would consist of two blood bank specialists trained to implement a bleeding activity and supervise its operation *in toto*. Equipment for the support of such an operation would be located at each area designated as a Blood Donor Center. Supporting personnel would be supplied by the medical installation at the bleeding site. The actual facility (gymnasium, etc.) location would be known by the various specialists. Following collection of the blood, the blood bank specialists would be responsible for its proper storage and designation.
3. The Blood Group Reference Laboratory of the US Army Blood Transfusion Service Center would serve as a central consultation activity for identifying blood group antibodies and assist in compatibility problems encountered in military blood transfusion services. There is no facility in the US Army providing this service for CONUS. The majority of blood grouping and crossmatch incompatibility problems (antibody detection and identification) encountered in military laboratories are sent to private and commercial laboratories. A few of the large commercial laboratories have one-week training and refresher courses in immunohematology. Numerous officers and civilian workers have attended these courses because it has been a means of keeping abreast of current changes and practices. It should be mentioned that commercial laboratories conducting refresher courses vary in the emphasis placed on recommended procedures. An example of this is use of Coombs serum, enzymes, and albumin reagents.
4. The flow of antibody problems and military personnel to the various commercial laboratories illustrates several significant areas to focus attention on. One is that there is a *void of technical activity* in this

Incl 1 to Annex E

field in the military services. Also training, standardization of methodology, and better communication in blood banking and immunohematology are in need of one of the following corrective actions:

a. Increase in training period at Medical Field Service School, Fort Sam Houston, Texas.

b. Change in subject material or emphasis.

c. Increase in training at the advanced level.

d. Availability of current standard methods.

e. Faster and wider dissemination of pertinent changes.

5. The referral of antibody problems to commercial sources does several things:

a. Drains valuable information (blood grouping data) and rare sera that otherwise would be available to the military through the Central Blood Grouping Laboratory.

b. Eliminates a source of direct information for compilation and reporting of significant findings by the military via the Central Blood Grouping Laboratory.

6. Finally, there is an urgent requirement for increased didactic training in blood banking and immunohematology and a need to increase the short, workshop-type laboratory courses in this field. This requirement for an active in-service training program is directly related to any attempt at standardization of techniques and technicians and to the maintenance of these standards once established. The US Army Blood Transfusion Service Center would have a Training Officer and Noncommissioned Officer responsible for training programs utilizing the Service Center staff as faculty for the following:

a. One-month program to train expert phlebotomists.

b. Three-month program to train blood bank technologists in all phases of blood banking.

c. Week-long workshops in immunohematology for refresher and advanced training for blood bank personnel Army-wide.

d. One-year blood bank fellowship program to train officers as directors of blood banks and officers of TOE Transfusion Service Units. Such a course would also serve as a source of highly qualified officers for the staff of the Transfusion Service Center.

7. Summary:

a. Activation of the US Army Blood Transfusion Service Center would be a major advance in establishing and maintaining higher overall standards in the Army's Blood Transfusion Service.

b. The US Army Blood Transfusion Service Center would be available to function as a testing or "back-up" laboratory in support of the ten functions assigned to the Department of Defense Military Blood Program Agency, Office of The Surgeon General, Washington, D. C.

c. Technical information gained from research, special studies, and operation experiences would be disseminated through proper channels.

d. Training of additional Blood Bank Officers, and military and civilian blood bank and immunohematology specialists would assist in coping with a current deficit of such personnel in the US Army.

US Army Blood Transfusion Service Center

Table of Distribution

ADMINISTRATIVE HQ
SECTION

Off 3

EM 5

Civ 14

CO

XO + Admin NCOIC

Tng Officer + Sgt

Data Processing Technicians - 3 Civ

Secretaries - 3 Civ

Supply Sgt + Civ

Shipping (1 EM + 1 Civ) 2

WB (Civ) 5 - Glassware

Janitor

Animal Care

Drivers (1 EM + 1 Civ) 2

BLEEDING OIC 1
TEAMS EM 5
Civ 3

LABORATORY OIC 1
SECTION EM 4
Civ 4

R & D Off 3 (2 MC; 1 MSC)
SECTION Civ 3

Collection
120 units/day

Processing
Blood Grouping
Crossmatch
Reference Problems

Research (Lab & Hosp)

Definition of parameters of
successful transfusion

Pathophysiology of adverse
effects of transfusion

R & D - Improved storage of
blood

Development - Equipment and
methods; system surveillance

OFF 8
EM 14
Civ 24

TOTAL 46

Incl 2 to Annex E

Detail of Civilian Personnel Assignments

ADMINISTRATIVE SECTION

Data Processing Technicians - must have knowledge of IBM data processing and statistics. GS-6 to GS-12

Secretaries - GS-3 to GS-6

Supply and Services - WB-5 to WB-7

Glassware - WB-3

Janitor - WB-3

Animal Care - WB-3

Driver - WB-4

COLLECTION

Bleeding Teams - 1 Registered Nurse - GS-9 to GS-11

2 Lab Technicians - GS-9

LABORATORY SECTION

Processing	}	Experienced immunohematologists GS-9 to GS-12
Blood Grouping		
Crossmatching		
Reference Problems		

R & D SECTION

MC slots may be occupied by MD's GS-13 to GS-15

Technical slots - GS-4 to GS-11

Major Items of Equipment

Glass washing equipment

Autoclave

Auto-technicon for blood grouping

Animal husbandry facilities

Standard laboratory equipment

Walk-in refrigerator

Jewett alarm and recording system

Ice making machines

Blood bank reefer mounted on truck

Blood mobile

-70°C refrigerator
IBM data processing equipment
Bleeding beds
Teaching machines
Standard office equipment
Animals (rabbits, goats, guinea pigs)
Refrigerated centrifuges
Liquid nitrogen storage facilities

Space

Full scale operation would require a minimum of 5,000 square feet.

Special Requirements

The US Army Blood Transfusion Service Center should be located with ready access to:

1. A large and constant turnover of the blood donor population such as a military basic training center for troops.
2. A Hospital Transfusion Service in operation.
3. A Hospital Clinical Service for clinical investigation.
4. Access to US Air Force transport facilities within a reasonable distance and to civilian air transport.
5. A Research and Development installation which could provide administrative and technical support and scientific collaboration.

Fort Knox, Kentucky, Fort Sam Houston, Texas, and The Presidio of San Francisco, California, meet these requirements.

Incl 3 to Annex E

Concept of Organization - Blood Group Reference Laboratory

1. Since antibody detection and identification will constitute the initial main workload of this laboratory, some facts are provided. A trained technologist can process eight bloods per day and test them in duplicate. Difficult problems would require retesting and referral to the chief technologist and supervising immunohematologist. A minimum of four technicians and an experienced supervising immunohematologist would be required for full operation of this phase.
2. The function of this very important phase of the Central Blood Group Reference Laboratory would include:
 - a. Identification of antibodies.
 - b. "Complete typing" facilities.
 - (1) A panel of red blood cells for antibody identification tests.
 - (2) A panel of red blood cells for transfusion purposes.
 - c. Preservation of samples - liquid nitrogen.
 - d. IBM Data Processing Facility for:
 - (1) Filing of data.
 - (2) Processing of information.
 - e. Antibody processing.
3. Two thousand square feet would house the reference laboratory and provide space for a clerk-typist and director.
4. Additional equipment required would include the following:
 - a. Liquid nitrogen storage facilities.
 - b. Special reagent cells and antisera.
 - c. Centrifuges (table models, refrigerated).
 - d. Deep freeze - walk-in type.
 - e. Refrigerators.

Incl 4 to Annex E

- f. Blood grouping and typing equipment.
5. The scope of functions of the reference laboratory would influence the personnel, equipment, and space requirements.
6. The US Army Blood Transfusion Service Center must maintain close liaison with the following:
- a. Defense Blood Program Agency.
 - b. Defense Medical Materiel Board.
 - c. Defense Supply Agency.
 - d. Division of Biologics Standards, NIH.
 - e. Walter Reed Army Institute of Research.
 - f. National Academy of Sciences, National Research Council.

Fort Knox, Kentucky

1. A ten-man team could initiate the operation of a Blood Transfusion Service Center at Fort Knox, Kentucky. The Army Medical Research Laboratory would serve as parent organization.
2. Nucleus of the team can be provided by transferring to Fort Knox the members of the Department of Immunohematology, WRAIR. Supplemental civilian personnel would be hired locally.
3. Building T-1027 of the Army Medical Research Laboratory contains an operational walk-in refrigerator. It was planned to set up in the building a laboratory of approximately 400 square feet. Renovation was begun and stopped. Laboratory furniture is in the building but not installed. Renovation could be completed and the furniture installed at an estimated cost of \$3,000.00. This would be adequate to begin operation of the Center on a limited scale.
4. The team would collect, process, and ship small quantities of whole blood, carry on a limited training program, perform reference work and small project studies. Data processing and research responsibilities would be assumed later upon expansion of unit.
5. Two available temporary buildings at Fort Knox can be reconstructed of cinder blocks at a cost of \$110,000 and would provide 5,000 square feet for the US Army Blood Transfusion Service Center. These buildings could be made ready for occupancy in approximately one year's time. During this time the initial team would study its mission "on the job," initiate recruitment, and assist in planning the reconstruction of the laboratory buildings.

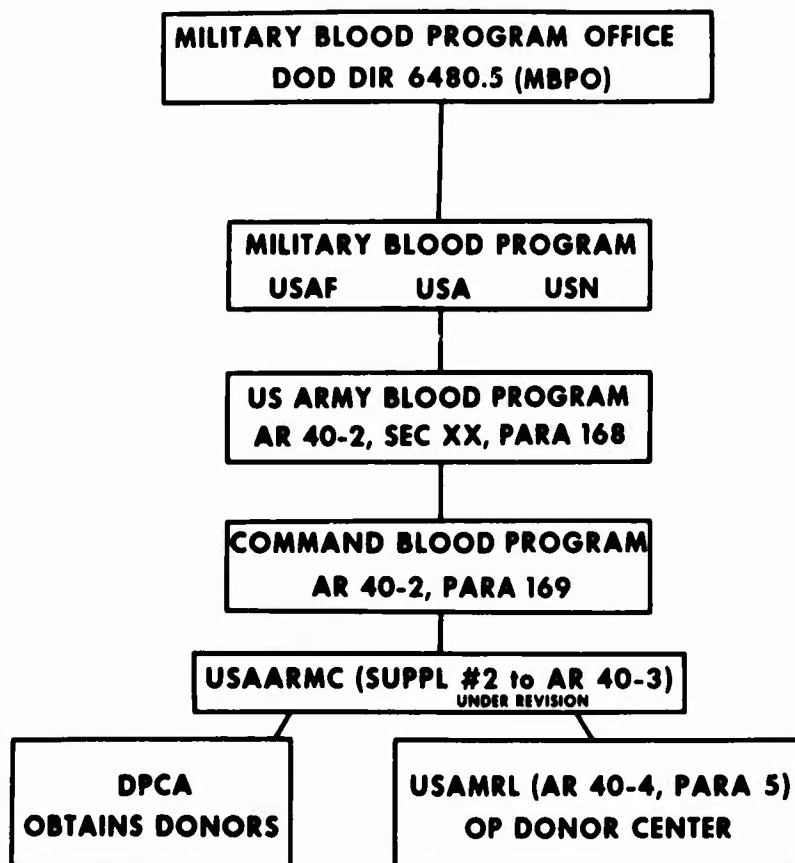
Incl 5 to Annex E

A N N E X F
C O N U S
ACTIVE MILITARY BLOOD DONOR CENTERS
Vietnam Blood Program

C O N U S

ACTIVE MILITARY BLOOD DONOR CENTERS

Vietnam Blood Program



Army

Fort Benning, GA
Fort Bliss, TX
Fort Bragg, NC
Fort Campbell, KY
Fort Devens, MA
Fort Dix, NJ
Fort Gordon, GA
Fort Hood, TX
Fort Jackson, SC
Fort Knox, KY

Air Force

Andrews AFB, MD
Beale AFB, CA
Barksdale AFB, LA
Chanute AFB, IL
Davis Monthan AFB, AZ
Dover AFB, DE
Eglin AFB, FL
Hill AFB, UT
Keesler AFB, MS
Lackland AFB, TX

March AFB, CA
Maxwell AFB, AL
Nellis AFB, NV
Offutt AFB, GA
Robbins AFB, GA
Scott AFB, IL
Shaw AFB, SC
Sheppard AFB, TX
Tinker AFB, OK
USAF Academy, CO

Fort Lee, VA
Fort Leonard Wood, MO
Fort Lewis, WA

Fort Ord, CA
Fort Polk, LA
Fort Sam Houston, TX
Fort Sill, OK

Langley AFB, VA
Lowry AFB, CO
McConnell AFB, KS

MacDill AFB, FL

Vandenberg AFB, CA
Westover AFB, MA
Wright-Patterson
AFB, OH

Navy

USNH Beaufort, SC
USNH Camp Lejeune, NC
USNH Camp Pendleton, CA
USNH Charleston, SC
USNH Great Lakes, IL
USNH Memphis, TN
USNH Orlando, FL
USNH Portsmouth, VA
USNH San Diego, CA

A N N E X G
BLOOD TRANSFUSION IN A PRISONER OF WAR CAMP

BLOOD TRANSFUSION IN A PRISONER OF WAR CAMP

An article* written by COL John E. Rogers, Jr., brings home a lesson "in doing what you can with what you have." During the summer of 1944, as prisoners of war (PW) of the Japanese, MAJ Rogers and a British Army physician scrounged and put together a *small transfusion service*. The equipment consisted of a pint Mason jar with a screw-on lid, an empty 1,000 ml double-ended bottle, a two-foot length of quarter-inch rubber tubing and an 18-gauge needle. A dasher was fabricated by an Australian PW tinsmith. Iodine crystals were obtained from the Japanese and rice alcohol was made in a still. Sterilization of equipment was accomplished in a deep instrument tray with boiling water. Cloth consisted of a sheet and mosquito netting. Blood from a donor whose dog tag ABO group was similar was tested for compatibility with the recipient's listed dog tag group. Using a glass slide (produced by the British physician from his personal gear), a drop of blood from each man was mixed together and observed from beneath the slide. A suspension of cells with no agglutination obvious to the naked eye was regarded as a compatible crossmatch. As the donor's blood flowed into the jar the dasher was turned constantly and slowly so that as the clot formed the strands of fibrin collected on the paddles of the dasher, leaving only serum and cells. Using this equipment and technic, eight or nine transfusions were performed in a prisoner of war camp along the Moulmein-Bangkok railway during World War II.

*Rogers, J. E., Jr. Primitive blood transfusion in a prisoner of war camp, W. W. II. *Milit. Med.* 131: 802, 1966.

A N N E X H
SECURITY MEASURES

Military blood banking has several aspects which require continuous monitoring and unique security measures. The following example is quoted from: Kendrick, D. B. Blood Program in World War II, Washington, D. C.: Office of The Surgeon General, Department of the Army, 1964, pp. 548-549.

"SECURITY MEASURES

"Unusual activity in the blood bank would, of course, have been a clear indication that the date of the invasion was approaching. On 1 May 1944, General Hawley wrote to the Commanding Officer, 1st Medical Laboratory (Memorandum, Maj. Gen. P. R. Hawley, to Commanding Officer, 1st Medical Laboratory, 1 May 1944, subject: Violation of Security), that the pony edition of *Time* for 24 April 1944 had carried an item to the effect that a recent dry run in the bank had been just for practice but that 3 weeks before the invasion, 'the dry run will become wet.' Obviously, General Hawley wrote, after such an announcement, no better indication could be given to the enemy of the date of the impending invasion than the inauguration of a stepped-up collection of blood. He found it necessary, therefore to direct that blood be collected on the maximum possible scale from this date until the invasion; otherwise, it would not be possible to resume collection until after the invasion. He requested all details concerning the origin of this statement and concerning the clearance of the particular correspondent responsible for it.

"In reply, Colonel Muckenfuss stated that no correspondent for this publication had ever visited the 1st Medical Laboratory; the term 'dry run' had not been used in the laboratory for at least 3 months; small-scale bleedings had been made at frequent intervals; and blood could not be kept longer than 3 weeks, which made the statement about beginning to collect blood 'in earnest' 3 weeks before the invasion obviously incorrect. He could therefore throw no light on the source of the statement. He added that he had discussed the problem of security several times with Major Hardin, to decide on methods of minimizing evidences of unusual activity in the blood bank.

"Immediately after General Hawley's complaint was received, all bleeding teams were sent out from the bank every day, to work all day and collect blood in places in which there were only a few donors, who were bled behind ostentatiously locked doors. At the end of each long day, the few donations thus procured were rushed in clearly marked 500-pint refrigerators to the blood bank.

"Actual blood collection for the invasion began 20 days before D-day, but, by Colonel Muckenfuss' own desire, he was not informed of Major Hardin's time schedule, and, as the latter expressed it in 1961,

'I was the only person who ever knew when the blood bank was actually turned on.'*

"*Queried as to the correctness of this statement, Dr. Hardin wrote as follows on 18 February 1963:

"'The statement that I made that I was the only person who 'knew when the blood bank in the ETO was actually turned on' is literally correct. The circumstances under which this arose now have somewhat unreal characteristics, but went something like this.

"'Several months before D-day the headquarters of the ETO blood bank was visited by a public relations officer who had in tow a *Time* reporter. Among the many questions asked of me was the one of how long blood could be kept. At that time the proper answer was 21 days and in due course there appeared an article in *Time* magazine which said the ETO blood bank would begin collecting blood 20 days before D-day. This was an assumption made by the reporter, but happened to be uncomfortably correct. As you can imagine, General Hawley was reasonably upset and he ordered me to undertake such activity as would make it impossible for people to know by observation when the ETO blood bank was actually 'turned on.' For that reason, we began somewhat hectic activity designed to produce confusion among all observers and among my own personnel. Bleeding teams were sent hither and yon, but always to units where there were too few donors to be of significance when we really started collecting blood. The blood was brought back to the central laboratory and processed and was distributed to hospitals so that there was no evidence at the central unit of how little blood was actually being collected.

"'The units of the ETO blood bank which were to go across the Channel were put into positions of embarkation along with other troops behind the barbed wire along the southern coast of England some time in advance of the invasion. They were sent there without instructions as to what their mission was or where they were going. Later I was given a pass which let me go behind the wire and brief my units and, as a matter of fact, take blood to them for transport across the Channel. As you know, we landed our first depot unit in Normandy on D-plus one. In addition, we loaded blood on 104 ships, most of which were LST's converted to bring troop wounded back from France. No one in the unit knew where these ships were to dock and be loaded, nor the day nor time, except myself and I kept this after receiving it at the British Naval Headquarters in Southampton entirely in my memory, never writing it down. I personally supervised the loading of refrigerator trucks in Salisbury and these and their drivers went behind the wire where they were met by some of my officers already in that locality. After accomplishing their mission these trucks and drivers were kept behind the wire until the invasion of Normandy was a fact.

"My memory fails me as to the exact time but early in the spring it became necessary for me to know when D-day would occur. One morning at General Hawley's headquarters in London, I was taken to the middle of a large room by Colonel Liston and others and the date of D-day was whispered in my ear. I was told that this date was a planning date and that the actual invasion would occur within a 48-hour span of this date. Thus I knew when to begin the bleeding in the blood bank in earnest, when to put blood behind the barbed wire along the southern coast, and when to begin all of the operation in earnest. I was forbidden to disclose this date to anyone else, of course, and although several of the people in the unit must have realized that D-day was imminent, I am certain that no one was actually aware of the real day until it happened.

"I hope this clears up my statement and I hope that none of us will ever go through that kind of an experience again."

A N N E X I
AABB CATEGORIES OF RESPONSE

AABB CATEGORIES OF RESPONSE*

Category I - Alert

When an individual bank is given an "Alert" category assignment, it should:

1. Inform its staff.
2. Ready donor recruitment, bleeding and processing mechanisms.
3. Insure availability of supplies and reagents for bleeding, processing, and shipping; take immediate steps to correct any deficiencies which might prevent it from functioning effectively under Category II, III, or IV assignments.
4. Establish close liaison as indicated under the circumstances with local police, Civil Defense, news media and communications agents; auxiliary communications arrangements should also be tested.

Category II - Inventory Expansion

When this category is established, there should be an immediate increase of donor blood stocks by 50% in preparation for further response category assignments. Call in and bleed donors. A predominance of groups A and O is recommended. The increased inventory should be maintained until notified to the contrary. These bloods should be used as needed, not held in reserve to avoid excessive outdating. As the situation develops, the bank may or may not be called upon to ship this blood. If not shipped, the bank is expected to utilize it in its regular operations. If necessary, the district office can assist the bank in locating an outlet for its surplus blood through the National Clearing House.

Category III - Immediate Shipment

Participating banks will be asked to contribute 50-90% of bloods available. The percentage of ABO groups, Rh types, etc., to be shipped will be specified in the disaster message. Bloods shipped should not be over 14 days old. This category of participation would be limited to a maximum of 3 consecutive days' effort.

Category IV - Sustained Production

Under this category, participating banks will be asked to contribute to the disaster program as well as to meet their ordinary local obligations. Fullest usage of available facilities is intended. The principal

* Disaster Plan Manual. American Association of Blood Banks. Chicago: Twentieth Century Press, Inc., 1st Ed., 1969.

extraordinary effort would be to recruit sufficient donors to maintain the full production capacity of the individual banks as they meet the needs of both their (regular) local obligations and the national disaster programs. The quantities, ABO groups, Rh types, and other matters will be specified, Bloods to be contributed should not be more than 4 days old.

A N N E X J

**MILITARY BLOOD BANKING
AREAS REQUIRING CONTINUED RESEARCH AND DEVELOPMENT**

**MILITARY BLOOD BANKING
AREAS REQUIRING CONTINUED RESEARCH AND DEVELOPMENT**

Coagulation Disorders in Combat Casualties.
Whole Blood Preservation (extension and improvement).
Oxygen Transport (red cell metabolism).
Universal Donor (group O) Studies.
Hemolytic Transfusion Reactions.
Transfusion Reaction Due to Other Causes.
Donor Identification.
Patient Identification.
Improved Plastics.
Automated Blood Grouping (quantitative hemagglutination).
Cryogenics - Frozen Red Cell Bank.
Fail-Safe Labeling.
Inventory System.
Transport, Packaging and Cargo Coding.
Hepatitis Screening.
Sickle Cell Screening.
Lymphocyte Typing (tissue typing) (tissue culture).
Gm Typing and Applications.
Antigen-Antibody Detection (immunohematology).
Standardization of Rh Nomenclature.
Blood Component Preparation, Transport, and Therapy.
Reference Laboratory for Problem Areas.
Blood Grouping Reagent Standards and Testing.
Forensic Testing.
Training for all Areas of Military Blood Banking.
Blood Bank Organization (improved).
Disaster Planning (updated).
Vaccines (A and B blood group substances).
Plasma Expanders (dextran, PVP, Hb, albumin, etc.).
Rare Donor Registry.
Bone Bank (tissue bank).
Organ (tissue) Transplant.
Storage, Refrigeration, Monitors (fail-safe systems).
Field Testing Laboratory (teams) for Problems in Military Blood Banking.
Improved Blood Filter to Eliminate Platelet and Other Debris Aggregates.
MTOE - Medical Table of Organization and Equipment.

A N N E X K

**MANAGEMENT IN MILITARY BLOOD BANKING FOR CONSERVATION
OF BLOOD RESOURCES: NEW ASPECTS CONCERNING
THE BLOOD DONOR BASE**

AD _____

REPORT NO. 964

MANAGEMENT IN MILITARY BLOOD BANKING FOR CONSERVATION
OF BLOOD RESOURCES: NEW ASPECTS CONCERNING
THE BLOOD DONOR BASE

(Progress Report)

by

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16 February 1972

Military Blood Banking: Methodology and Equipment
Work Unit No. 158
Combat Surgery
Task No. 00
Combat Surgery
DA Project No. 3A062110A821

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ABSTRACT

MANAGEMENT IN MILITARY BLOOD BANKING FOR CONSERVATION
OF BLOOD RESOURCES: NEW ASPECTS CONCERNING
THE BLOOD DONOR BASE

OBJECTIVE

To review the changes in blood bank management in the past 36 months; and to ascertain how new technology can counterbalance certain aspects concerning the blood donor base.

APPROACH

The Blood Bank Center of the US Army Medical Research Laboratory, Fort Knox, Kentucky, is used as a model of operations upon which to evaluate new technology in blood bank management.

CONCLUSIONS

The status of military and civilian blood banking is discussed from several viewpoints. The progress made in collecting, processing, storage, and clinical use is reviewed from 1940 to 1972. Several major obstacles have been either eliminated or substantially improved. Blood component therapy has changed the overall operation of blood banks in a remarkable manner. It has now made possible the use of multiple management techniques, heretofore not possible, due to the short shelf life of whole blood. These management techniques include: economics, accounting, mathematics and statistics, and the behavioral sciences. The gross waste of red blood cells tolerated until recently is no longer existent; nor is the commercial plasma salvage technique the only outlet for whole blood not used for patients. The transition to the newer operation of blood component preparation has not come too soon. Our blood donor base, which represents a critical national resource in peace and war, is being eroded by drug abuse, hepatitis carriers, sickle cell trait and disease, malaria, and immunizations. This is coupled with donor apathy, rising cost of blood bank operations, and the problems inherent in recruiting, training, and retraining qualified blood bank personnel.

Procedures are provided for the preparation of several components followed by a discussion of their usefulness and precautions.

In conclusion we must recognize that with more efficient production, testing, and use of specific components, implementation of sound management principles, increased training programs, and continuing research and development--proper conservation of our blood resources can be achieved. Expansion of frozen blood banking undoubtedly will make a significant contribution toward this goal.

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MANAGEMENT IN MILITARY BLOOD BANKING FOR CONSERVATION
OF BLOOD RESOURCES: NEW ASPECTS CONCERNING
THE BLOOD DONOR BASE

INTRODUCTION

Recent developments in civilian and military blood bank practice have brought about changes in daily operations, such that in 1972 *all management techniques* can now be practiced. This was not possible a few years ago.

Broadly speaking, we are referring to master guidelines (1,2) which include:

1. Managerial economics.
2. Managerial accounting.
3. Mathematics and statistics.
4. Behavioral sciences.

We consider management as a process through which a well-oriented group or team concentrate efforts to achieve required objectives.

REVIEW OF BLOOD BANK CHANGES

The decade 1940-1950 witnessed the development and adoption of techniques to preserve whole blood for 24 hours, 5-6 days, 10 days, and finally, 21 days. World War II provided the impetus to research as well as use of preserved blood. The principles of acidification, refrigeration, and the addition of dextrose were adjusted to maintain the proper pH and essentials for red cell metabolism *in vitro*. From these studies acid-citrate-dextrose (ACD) became the preservative and anticoagulant of choice.

The second major change occurred in 1950-1960 when plastic blood bags were introduced during the Korean War; their logistic superiority over glass bottles was demonstrated during this time. Shortly thereafter, satellite bags were attached to the blood collection bag and separation of blood components was possible for the first time--in a closed (sterile) system (3,4). At about the same time, another preservative was being studied--citrate-phosphate-dextrose (CPD)--and other studies were being conducted with substances such as adenine and inosine to extend the shelf life of preserved red cells.

The third major change occurred during 1960-1970 when the following advances were either introduced, reported, or expanded (5-7):

Plasmapheresis (8-11).

Frozen blood banking (12).

Blood component separation and therapy (13).

Preparation of cryoprecipitate and other concentrates (14-17).

Reports concerning addition of adenine to whole blood (18-20).

The role of 2,3-diphosphoglycerate (2,3-DPG) in the metabolism and function of mammalian red cells--oxygen transport.

CURRENT SITUATION

Fluctuations were experienced in maintaining adequate inventories of whole blood in 1971. This hard fact confronts civilian and military blood banks alike. The reasons for this are rather persistent:

1. Holiday: shortages of donors.
2. Holiday: increase in accidents.

The tools of management would seem to be the answer to the current dilemma and, indeed, this is the case. Prior to the period 1965-1968, only a few of the management tools could be used effectively to assist in overall blood bank management. Specifically, we are referring to sufficient inventory to properly meet blood requirements while at the same time avoiding costly waste in terms of money and loss of a valuable resource. In a system employing essentially whole blood, the use of expensive computer systems can only reduce waste to a certain degree. Expiration dates remain the ultimate factor controlling waste. Some would argue that plasma salvage resolves whatever problem that exists in this area. We disagree totally on this viewpoint and present the following short résumé of a local experience.

In analyzing the plasma salvage operation in 1966 and 1967, it was quite apparent that a substantial portion of the blood collection and processing effort *was not going to military hospitals for transfusion therapy*, but rather to industry (plasma) for fractionation; whereas red blood cells, not used by transfusionists (who were yet unaware of their value in many clinical situations) went down the drain.

To halt this waste of a valuable national resource (blood) and to reap a fuller yield from each unit of blood, separation of blood into its various components in a closed plastic system was initiated. The American Association of Blood Banks then began an intensive training program in all phases of blood component preparation and therapy. This included workshops at the annual meeting, training films, and a manual on blood components which was made available to all professional personnel in blood banking. In 1968, 1969, and 1970, the American Association of Blood Banks, with assistance from the National Institutes of Health, conducted blood component workshops in large cities throughout the United States.

At the Fort Knox Blood Bank blood component preparation began, at first moderately and then explosively; the same momentum was being experienced in the large blood banks of the civilian community. Blood component preparation had arrived and the impact is still being evaluated for better management of future operations. The pioneers in this exciting new field of medical care experienced all the hardships encountered in any uncharted course of action. *Success* in blood component preparation and therapy, however, has removed all of the early obstacles. Effort is now concentrated in improving present methodology (Fig. 1).

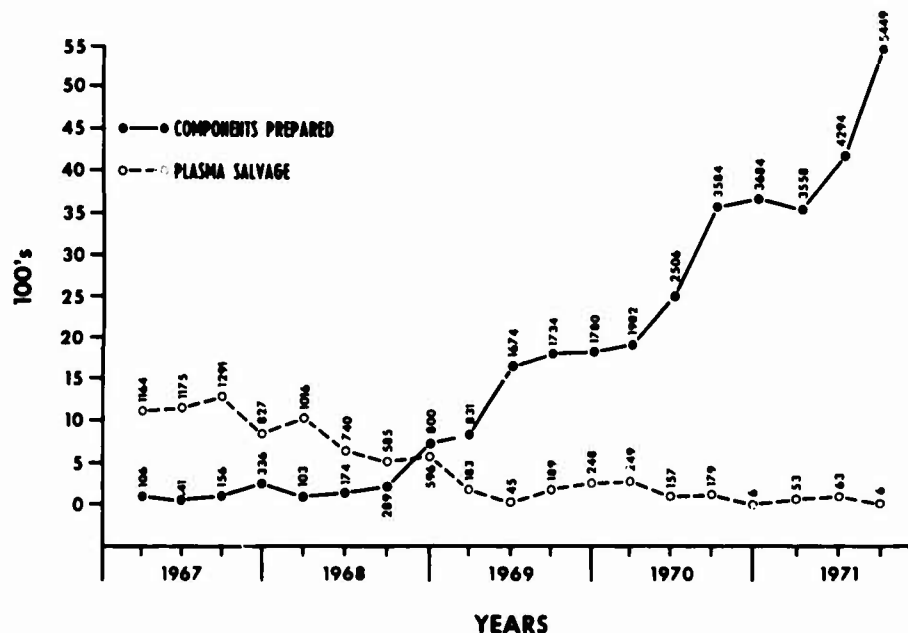


Fig. 1. Plasma salvage vs. components prepared.

CURRENT OPERATION

Presently the blood bank operation at Fort Knox produces the following products:

1. Whole blood.
2. Packed red blood cells.
3. Platelet-rich plasma.
4. Platelet concentrate.

5. Fresh frozen plasma.
6. Cryoprecipitate.
7. Reconstituted whole blood.

This wide spectrum of components serves two important purposes:

1. Improved blood transfusion therapy.
 - a. Prevention of circulatory overload.
 - b. Selection of component required for the patient.
 - c. More efficient utilization of donor blood.
 - d. Treatment of coagulation defects.
 - e. Less chance of isoimmunization.
 - f. Smaller dose of hepatitis virus with packed cells (21).
2. Improved blood bank management.
 - a. Sound inventory practice.
 - b. Increased productivity.
 - c. Flexibility.
 - d. Better control of outdating.
 - e. Reserve stockage for routine and emergency use.

Several key aspects make it possible for improved blood transfusion therapy and management. These are:

1. Plastic blood bags.
 - a. Closed system.
 - b. Prevention of bacterial contamination.
2. Refrigerated centrifuges.
3. Cascade type freezers.
4. Walk-in refrigerators.

BLOOD COMPONENTS AND THEIR PREPARATION

Whole blood transfusions have been the mainstay of transfusion therapy since World War II. Yet, during World War II, the most commonly used blood component was plasma. Though valuable for volume expansion, plasma lacked the oxygen carrying capacity of the erythrocytes. Now, after two decades, the value of separate therapeutic agents is being realized. In fact, the majority of certified blood banks feel that a versatile program should contain not only the packed cell and plasma portions but these should be further subdivided to provide highly potent and medically useful components for such problems as hemophilia, leukemia, and thrombocytopenia.

Fractionation of plasma into constituents such as albumin, fibrinogen, gamma globulin, glycine precipitated AHG, and Factor II-IX complex requires more sophisticated equipment and, therefore, is not part of the routine hospital blood bank operation. However, blood banks are the sources for the pooled plasma later used to produce these specific fractions. Facilities for routine separation of the major components, such as those located at the Blood Bank Center, US Army Medical Research Laboratory, Fort Knox, Kentucky, can be implemented in any certified blood bank (Figs. 2-5).



Fig. 2. Building 1006, Processing Laboratory.



Fig. 3. Front office, Building 1006.



Fig. 4. Laboratory.



Fig. 5. Preparing boxes for shipment.

Despite the general interest in blood component preparation, there is a lack of standardization in both preparation and storage. The blood component operation developed at the Blood Bank Center was created to support military blood banks in the Continental United States and overseas. The procedures used in this operation are described below.

The double and triple BLOOD-PACK[®] units of Fenwal Laboratories (Fig. 6) and the Sorvall RC-3 general purpose automatic refrigerated centrifuge with a horizontal head (HG-4) (Fig. 7) are used for component preparation at this center. Special features of the centrifuge include quick acceleration, high speed, automatic temperature control, and acceptance of a 5 gram imbalance that make it an ideal instrument for rapid and safe processing of blood components.

In all of the procedures outlined below, the opposed centrifuge cups are balanced with blood bags on a Model PL-12 torsion balance using rubber balancing discs. Polyethylene overwraps are used on the bags (Fig. 8).

In collecting blood for component preparation, the arm is prepared as for any other blood donation using the alternative method outlined in the AABB Technical Methods and Procedures, 5th Edition (22). Care must be taken in performing the phlebotomy to insure minimal tissue trauma, an uninterrupted flow of blood, and constant, gentle, thorough mixing. This technic is followed to prevent the initiation of the clotting mechanism which, when activated, will decrease levels of some of the clotting factors such as Factor VIII (AHF) and Factor III (platelets).

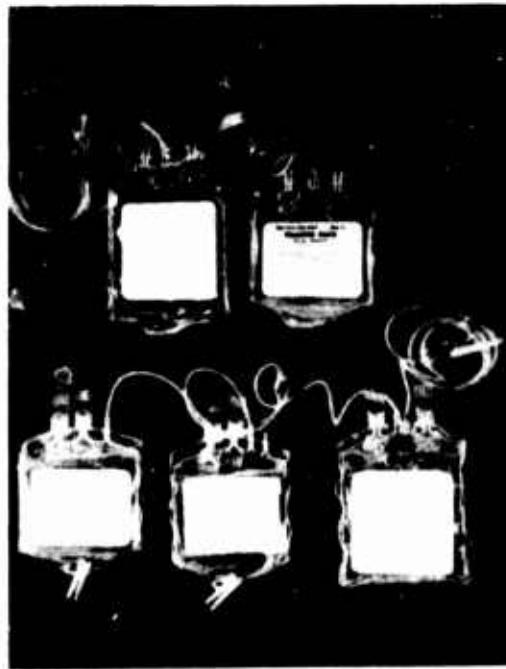


Fig. 6. Double and triple blood bags.



Fig. 7. RC-3.



Fig. 8. Torsion balance.

The following component preparation methods used at the Blood Bank Center are patterned after those set forth by the AABB, but will deviate slightly in technic and centrifugation times for certain products.

1. Packed Red Blood Cells and Fresh Frozen Plasma. Packed red blood cells and fresh frozen plasma are prepared by using the double unit blood pack with ACD (Fenwal 4R102). The blood is collected into the larger bag with careful attention to thorough mixing of ACD and blood. Within 2 hours of the collection, both bags are prepared as described above and centrifuged 4200 RPM (4900 RCF) at 4-8 C for 10 minutes. The time of spin starts when the centrifuge is turned on and not when the desired speed has been reached. The centrifuged unit of blood is placed on a plasma extractor and the satellite bag on a dietary scale (Fig. 9). The dietary scale is set at zero and 220 ml of plasma are expressed from the unit. The tubing is clamped and segments for future use are made with the dielectric sealer before the tubing is cut (Fig. 10). The bag with the plasma is appropriately labeled and placed in a freezer at -60 C and is usable for 1 year as fresh frozen plasma (Fig. 11). The packed red blood cells having a hematocrit of approximately 70 vol percent is appropriately labeled (Fig. 12) and placed in the storage refrigerator at 2-4 C. The packed red blood cells, prepared in a closed system, have the same 21-day expiration date as whole blood.



Fig. 9. Dietary scale.

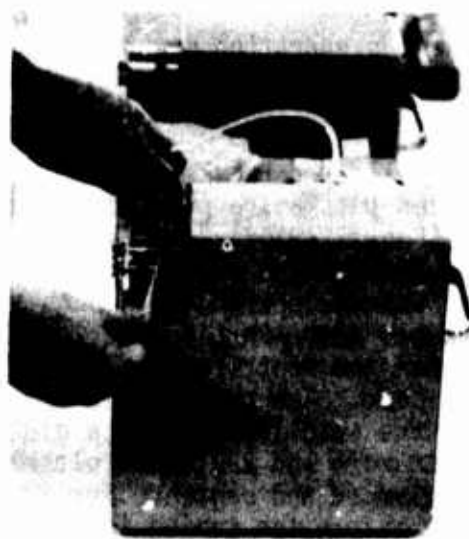


Fig. 10. Dielectric (ler.



Fig. 11. Freezer.



Fig. 12. Labeling.

2. Platelet-Rich Plasma and Platelet Concentrates. Double unit ACD blood packs are used in obtaining blood. They are collected and prepared for centrifugation, as previously stated, and centrifuged within 1 hour of collection. The temperature is maintained at 22 C while centrifuging at 3600 RPM (3630 RCF) for 2-1/2 minutes. The unit of blood is then placed on a plasma extractor and the satellite bag on a dietary scale. The scale is set at zero and 220 ml of plasma are expressed from the unit. (If platelet concentrates are to be made and the plasma returned to the original blood bag, more plasma may be removed, resulting in a better platelet yield.) The tubing is then clamped and segments for future testing are made with the dielectric sealer before the tubing is cut. The bag is appropriately labeled and contains 220 ml of platelet-rich plasma. When platelet concentrate is prepared, the platelet-rich plasma in the satellite bag is left attached, with tubing unsegmented, to the original bag containing the packed red blood cells. After preparation for centrifugation, they are centrifuged 4200 RPM (4900 RCF) at 22 C for 5 minutes. All but 25 ml of plasma is then expressed back into the plastic bag with the red blood cells. The original blood bag is labeled according to AABB standards showing that platelets have been removed and can be used up to 21 days from date of collection.

The transfer pack containing the platelet button and 25 ml of plasma is incubated at room temperature for at least an hour before resuspending. Quality control tests are performed each day of preparation to check on the efficiency of centrifugation and technics. Both platelet-rich plasma and platelet concentrates are maintained at room temperature (22 C). Platelets are maintained and shipped at this temperature with or without ice (depending on the environmental temperature) in styrofoam containers to the distant using military facilities and are available for use within 6-8 hours of collection. The platelets are used in most cases up to a maximum of 48 hours for optimal clinical results.

The triple unit ACD blood packs are used also in platelet concentrate preparation. When these are used, cryoprecipitates are made from the platelet-poor plasma before the whole blood is reconstituted.

3. Cryoprecipitate (Factor VIII) and Whole Blood with Cryoprecipitate Removed. A double ACD blood pack is used in the collection of blood as described previously. The unit is prepared for centrifugation and centrifuged 4200 RPM (4900 RCF) at 4-8 C for 10 minutes as outlined in number 1, above. The centrifuged unit of blood is placed on a plasma extractor and as much of the plasma as possible, without mixing the red blood cells, is expressed into the satellite bag. The tubing is temporarily clamped. The satellite bag with the plasma is inserted into a polyethylene bag and partially submerged into a 95 percent ethanol-dry ice mixture at approximately -60 C until frozen solid (Fig. 13). This usually takes 15-30 minutes. The plastic bag containing the red blood cells is placed on a large plastic bag containing wet ice during the plasma freezing time.



Fig. 13. Freezing bath.

The frozen plasma and red blood cell units are placed in a walk-in blood bank refrigerator at 2-4 C. Care should be taken to keep the plastic bag containing the red blood cells from touching the freezing mixture or the frozen product and also to place them on a shelf below the plasma in the refrigerator (Fig. 14). The plasma is allowed to thaw overnight and the units prepared for centrifugation in the usual manner. They are centrifuged 4200 RPM (4900 RCF) at 4-8 C for 10 minutes.

The plasma unit is hung upside down and all of the plasma drained into the unit containing the red blood cells (Fig. 15). The cryoprecipitate (Factor VIII) remains in the bottom of the plastic bag. The bag is then labeled appropriately and stored in a freezer at -60 C and is usable for 1 year from processing date. The whole blood with cryoprecipitate removed is also labeled according to AABB specifications and is usable for 21 days from collection date.



Fig. 14. Walk-in refrigerator.

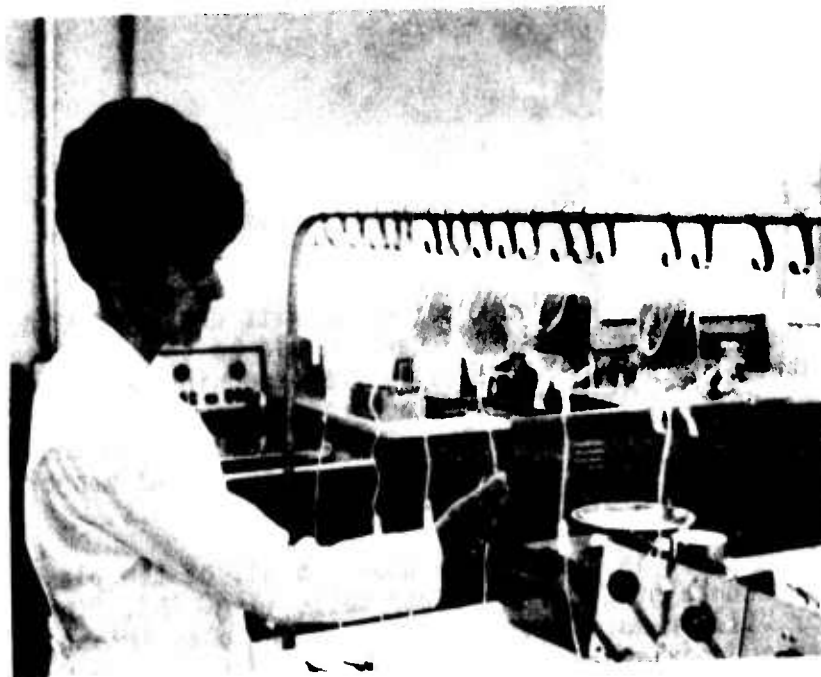


Fig. 15. Draining plasma.

COST ANALYSIS OF BLOOD COMPONENTS OPERATION

In this section processing costs per component unit will be reviewed (Fig. 16).

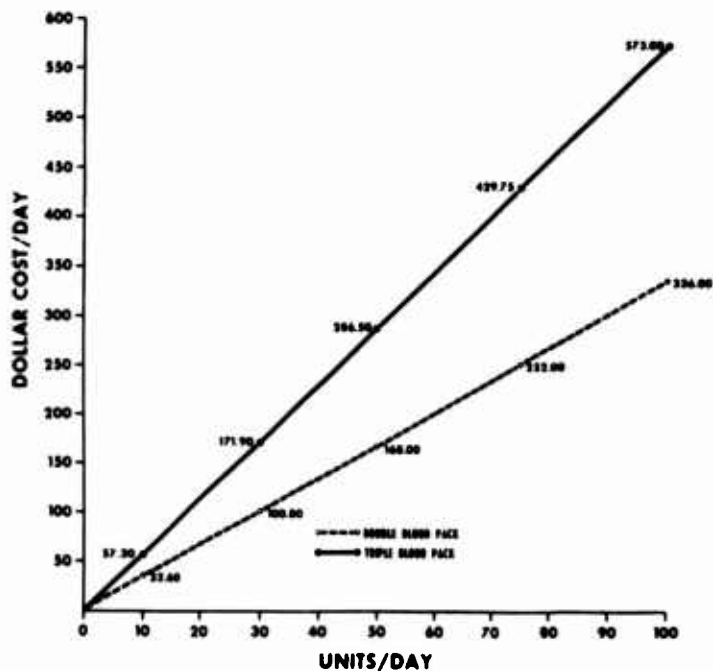


Fig. 16. Component preparation average cost/day.

When blood component therapy is studied in depth, preparation of all products may be considered under four basic procedures. Each component is developed through a rapid succession of all or part of these four steps. Generally, these four steps include:

1. Centrifugation--to include bagging, weighing, balancing, and placement into centrifuge. This step is used repeatedly throughout preparation.
2. Extraction--expression of plasma.
3. Quick freeze and thaw--a freeze at -60 C and then a thaw overnight at 4 C.
4. Preparation for final freeze--to include segmenting, labeling, and quality control.

It is a relatively simple matter to study preparation of individual or bulk units. Information readily available concerns number of units produced, number of people involved, time required to produce each unit, and equipment depreciation costs. Due to the excessive cost for equipment and labor, only a large blood facility can economically produce components in volume. Cost estimates were based on a short-term survey under actual processing conditions. Component production teams of three and four technicians were used; labor was costed as an hourly average.

During the course of the study, 50 units were processed per day. Surprisingly, the costs per unit for greater volumes were not reduced. It was noted, however, that labor costs were approximately 20¢ less per unit when the triple blood pack rather than the double pack was used.

Costs incurred while typing whole blood samples have been evaluated by Forrester, Shields, Camp, and Harville (23) for both manual and automated systems. This study was not repeated during the component review. Whole blood costs should be added to component costs for a complete evaluation of donor center processing expenses. *Factors not considered in this study included building depreciation and overhead personnel costs (secretarial, janitorial, etc.).* These elements are relatively fixed in relation to a nonprofit military blood center, regardless of component preparation volume. It should also be noted that centrifuge spin, freezing, and thawing times were excluded when technicians were busy with other production tasks.

The average production cost of one particular component, at a volume of 50 units per day, was as follows:

	<u>Double blood pack</u>	<u>Triple blood pack</u>
Packed cells	\$3.19	\$5.35
Plasma (fresh frozen)	3.19	5.35
Platelet-rich plasma	3.19	5.35
Cryoprecipitate	3.61	5.96

The cost of whole blood and components varies from area to area within the United States. A community type blood bank in a large northeastern city has the following charges:

<u>Product</u>	<u>Cost per unit</u>
Whole blood	\$15.00
Packed red blood cells	10.00
Cryoprecipitate* (100 AHF units)	10.00
Platelets (concentrate or platelet-rich plasma)	10.00
Fresh frozen plasma	10.00

* Commercial cost is \$80.00/vial, which is equal to 500 AHF units.

Single donor and double-pack units are currently standard items in military supply. It is anticipated that the triple-pack unit will also become a standard item by early 1972, with a concomitant reduction in cost.

In an era of increasing demand and decreasing supply, the value of an additional component from one unit of whole blood readily compensates for the increased cost of using triple blood packs.

Figure 17 shows a reduction in the number of units of whole blood collected from 64 per 100 donors processed in 1970, to 57 percent in 1971.

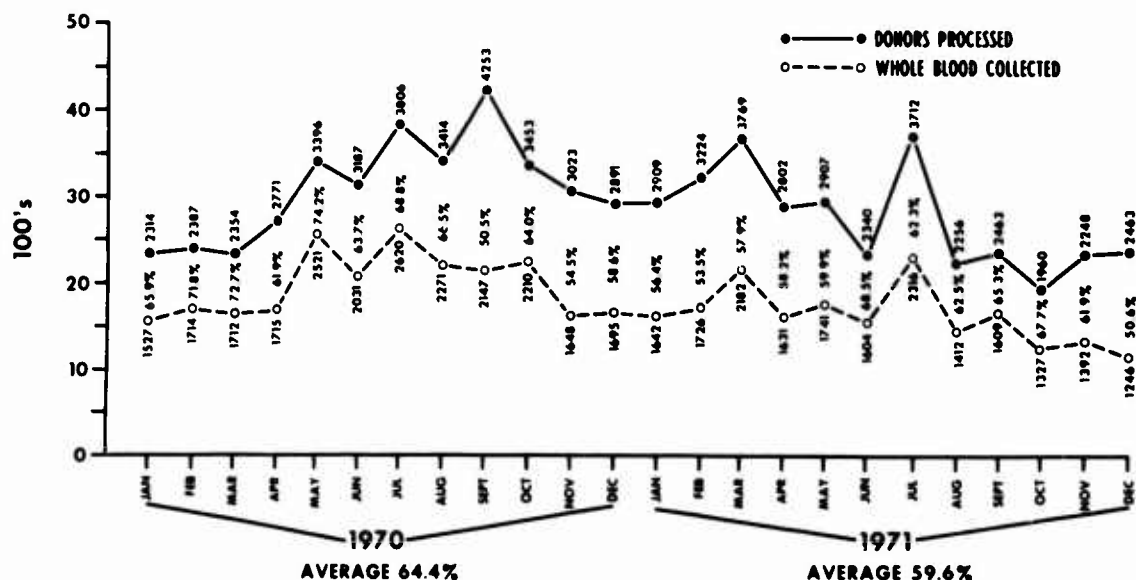


Fig. 17. Donors processed vs. collected.

On the other hand, the number of blood components prepared and shipped is steadily increasing in seemingly geometric proportions (Fig. 18). It is interesting to note that the projected shipments beyond April 1971 (broken line) were exceeded by the actual shipments. Also, it is significant that constraints must be placed on component users to the extent that a request for 300 units of cryoprecipitate is reduced to 200; the requesting hospital is advised to keep the Fort Knox Blood Bank Center alerted if the situation warrants immediate and continued support. Often there is a requirement for sustained support, as in the case of a hemophiliac undergoing surgery.

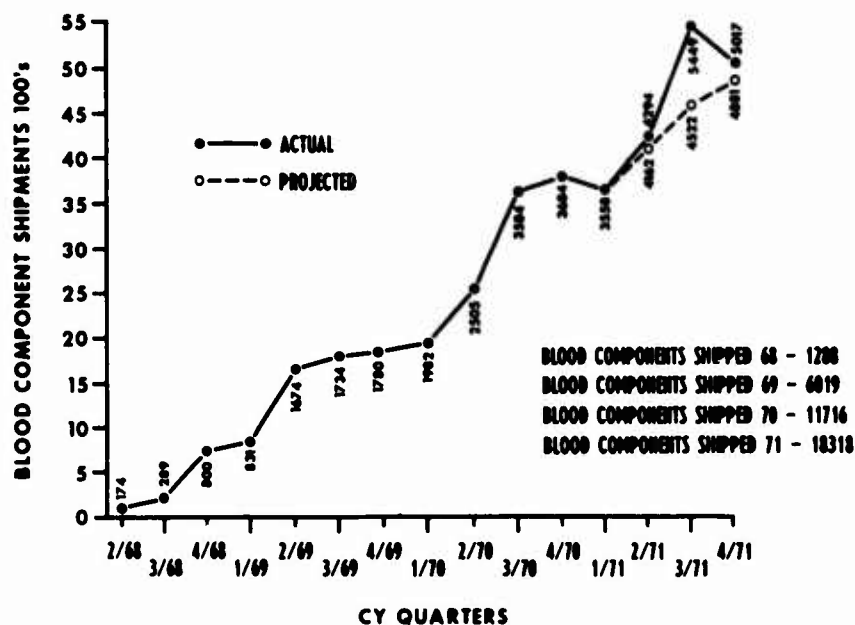


Fig. 18. Blood components shipments by quarter.

DISCUSSION

1. Improved Blood Transfusion Therapy. Diagram 1 is used to illustrate some of the components and their usefulness (24).

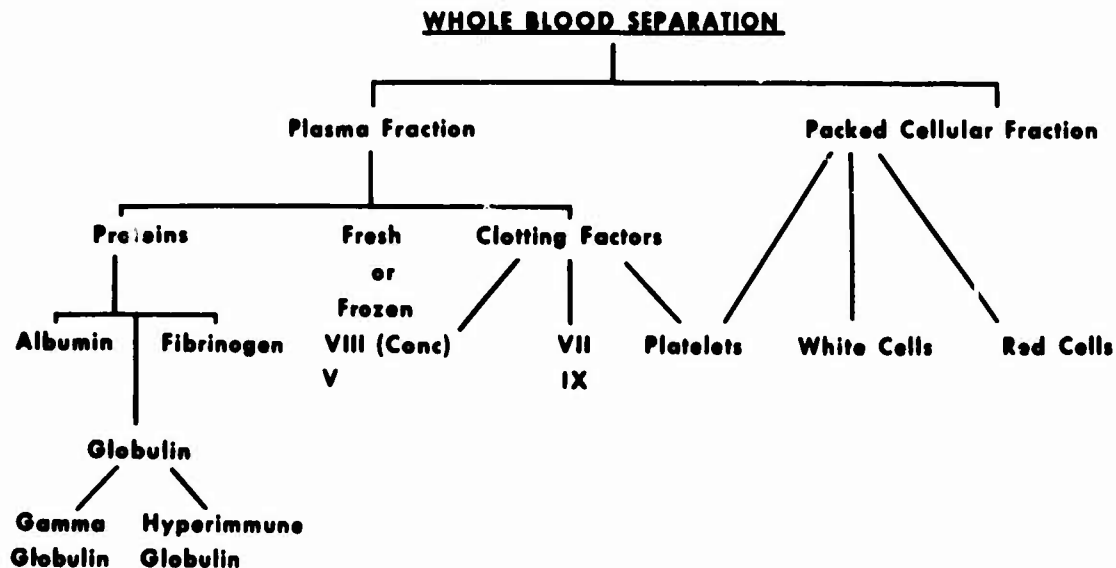


Diagram 1.

Freshly drawn blood can provide all known clotting factors in plasma, including platelets. Therefore, it is considered the universal emergency therapy for bleeding disorders. A glycine-precipitate method for concentrating antihemophilic factor (Factor VIII) may supply a more potent and specific component and reduce the risk of developing antibodies to this factor.

In protein separation, basic fractions consist of albumin, globulin, or fibrinogen. Albumin has value as a plasma expander, in the treatment of burns, nephrotic syndrome, pancreatitis, cirrhosis, hypoproteinemia, and for its nutritional value when needed. Fibrinogen is useful in specific deficiencies whether congenital or acquired but carries the risk of hepatitis transmission. The globulin fraction can be used as prophylaxis for many infectious diseases, including exotic diseases and hepatitis, and as specific replacement therapy in globulin deficient states. Additional plasma subfractions can be obtained from commercial firms and include plasma (fibrinolysin), ceruloplasmin, plasma cholinesterase, siderophilin, and highly specific immunoglobulins protecting against measles, mumps, pertussis, and tetanus.

The critical demand for gamma globulin and other blood fractions available only from human plasma has emphasized the need for an efficient plasma salvage program. The recognition of the lability of various portions has been the stimulus for programs designed to procure fresh plasma either by the immediate separate method as described, or through plasma-pheresis. Although the demand for plasma is sufficient reason to use packed red blood cells whenever possible, packed cells frequently have a distinct advantage when transfusions are primarily concerned with providing the oxygen transport function of red blood cells and circulatory overload may pose a problem.

The separation process also permits adjustment of the concentration of the various components; for example, providing platelet-rich plasma or packed cells, or leukocyte-poor whole blood or red blood cells may be more effective therapeutically in certain conditions and may obviate the risk of some of the adverse reactions of whole blood transfusion.

Hemophilia and Fresh Plasma Therapy. Fresh frozen plasma provides a means of furnishing an increase in the patient's circulating plasma. Factor VIII is essential for the hemophiliac with soft tissue and joint bleeding, not readily controlled by local measures. When anemia and anoxia are not critical, treatment with fresh plasma is specific.

Cryoprecipitate. Cryoprecipitate offers a higher concentration of Factor VIII for the hemophiliac. The use of cryoprecipitates has revolutionized the treatment of hemophilia in recent years. The principal limitation for its use has been the availability of the starting material, fresh frozen plasma. However, with a proper level of packed red cell usage in the community and some centralization of blood component production, there should be an adequate supply of fresh frozen plasma.

The dosage of cryoprecipitates varies with the severity of hemophilia and type of injury. Hemophilic patients should have the benefit of periodic Factor VIII assay to determine the efficacy of treatment. In general, 1 unit/10 kg of body weight is required to produce a significant rise in Factor VIII level, repeated every 12 hours until hemostasis is assured. Smaller doses may be utilized when bleeding is not major or if hemophilia is not severe.

Because only a small amount of plasma is given with each cryoprecipitate unit, crossmatching is neither necessary nor desirable. The cryoprecipitate should be given on a type-specific basis if possible. Small doses of incompatible plasma are usually not dangerous except in young children, but when plasma dose begins to increase, even adult recipients may begin to exhibit signs of hemolytic anemia due to the presence of transfused incompatible isoagglutinins (25,26).

Platelets. Platelet transfusions are clinically useful; the criteria of effectiveness are usually platelet counts and cessation of bleeding. In the last analysis, however, the value of platelet transfusions is based largely on clinical judgment and experience. *In vitro* measurement of platelet functions does not serve as a useful index of clinical effectiveness. For example, Cronkite states that, in general, bleeding appears at higher platelet levels when thrombocytopenia develops rapidly than when the process is chronic. Thrombocytopenic bleeding may be controlled by the transfusion of viable platelets although quantitative evaluation of effectiveness is very difficult. Measurement of the life-span of transfused platelets may be linear or exponential but the ideal method for performing such studies has yet to be determined (27).

Freireich, et al, have reported that in patients who have developed platelet isoagglutinins, plasmapheresis of a compatible platelet donor is an alternative solution. Probably the most encouraging development is platelet survival up to 96 hours after bleeding the donor, if they are stored at room temperature (28-31). However, contamination demands careful processing of platelet preparations (Tables I and II).

Hemoglobin Function. The ability of red cells to release oxygen to the tissues depends on the metabolite, 2,3-diphosphoglycerate (2,3-DPG). Research in several laboratories has indicated that 2,3-DPG, and thus hemoglobin function, can be maintained in red cells for the entire storage period (3-4 weeks with ACD and CPD or 5-6 weeks with adenine and inosine) if metabolism is regulated by changing the acidity of the preservative or including pyruvate or methylene blue (32).

2. Improved Blood Bank Management. The present day blood bank manager is faced with very unique problem areas. Let us take a microscopic view of factors that are currently playing major roles in their effect on his "blood donor base." These factors include (Fig. 19):

TABLE I

SOME HEMORRHAGIC DYSCRASIAS AND THEIR TREATMENT*

International Nomenclature	Synonyms	Treatment of Deficiency
I	Fibrinogen	Fibrinogen concentrates
II	Prothrombin	Whole blood; plasma (up to 3 weeks old)
V	Proaccelerin, labile factor	Whole blood; plasma (up to 7 days old)
VII	Proconvertin	Whole blood; plasma (up to 7 days old); fresh-frozen plasma
VIII	Antihemophilic factor	Whole blood; plasma (less than 24 hr old); fresh-frozen plasma
IX	Christmas factor	Whole blood; plasma (up to 7 days old); fresh-frozen plasma
X	Stuart factor	Whole blood; plasma (up to 7 days old); fresh-frozen plasma

*From Grove-Rasmussen, Lesses, and Anstall (34) as modified by Sturgeon, et al (33).

TABLE II

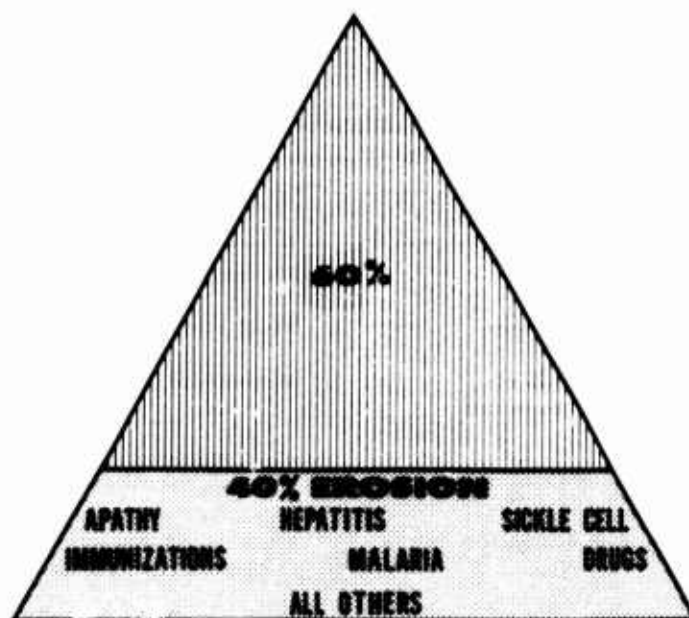
PLASMA PROTEIN FRACTIONS EMPLOYED
IN THE TREATMENT OF BLEEDING DISORDERS*

Disorder	Preparation	Manufacturer
Classical hemophilia	Cohn fraction I Fraction I-O Glycine precipitate Ether precipitate Cryoprecipitate AHF concentrate	Blomback Wagner Kekwick Pool Hyland, Courtland
Hemophilia B	P. P. S. B. ** Prothrombin complex	Soulier Konyne***, Cutter
Von Willebrand's disease	Cryoprecipitate	Pool

*From Sturgeon, et al (33).

**P. P. S. B. = Prothrombin, proconvertin, Stuart, and antihemophilic B.

***Konyne - Registered Trademark.



1972 ENTRY BLOOD DONOR

Fig. 19. Blood donor base.

- a. Drug abuse.
- b. Hepatitis.
- c. Sickle cell.
- d. Donor refusal (apathy).
- e. Immunizations.
- f. Other donor rejection criteria (e.g., malaria, etc.).
- g. Rising cost of operations.
- h. Recruitment of personnel.
- i. Training of personnel.
- j. Retention of personnel.

HUMAN RELATIONS

As blood component preparation became a larger part of the blood bank operation, new avenues of growth presented themselves for both the Blood Bank Center as a whole and for individual members of the staff.

The change in activity affected supply procedures, collection of blood, processing, storage, and shipping practices. New guidelines and procedures for blood component preparation were introduced and distributed to all members of the staff. Training classes and demonstrations were held. Certain individuals were given direct responsibility for certain aspects of the blood component operation. When the serious problem of high incidence of breakage of fresh frozen plasma and cryoprecipitate was brought to everyone's attention, *the problem was essentially resolved by the staff.* The application of ingenuity, creativity, and imagination led to the use of a special air cap plastic material which cushioned the fragile units better than anything available on the commercial market. The importance of this contribution made possible shipments of fresh frozen plasma and cryoprecipitate to areas of military exigency in Southeast Asia (Fig. 20). This is the first time blood components have been widely used in military medicine during combat.

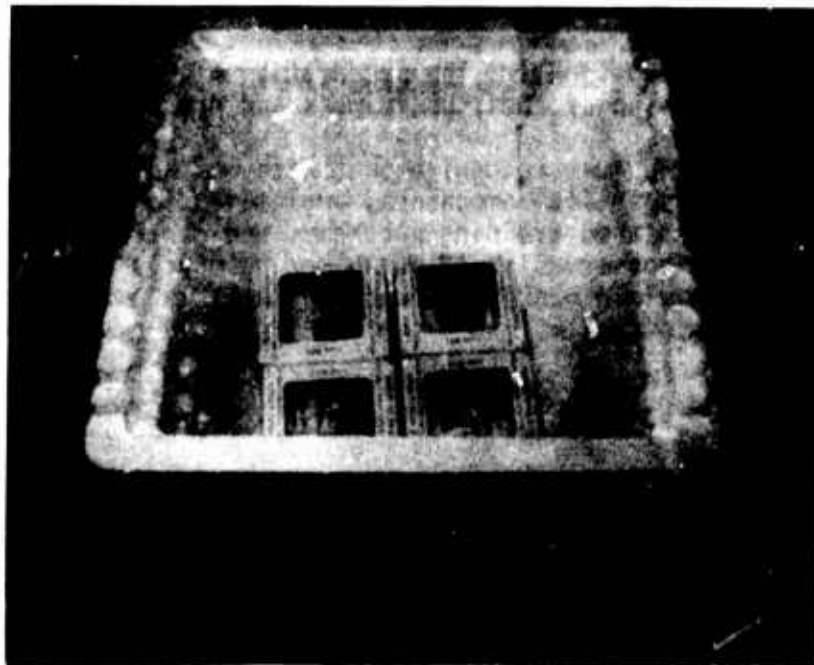


Fig. 20. Aircap.

Sturgeon, et al, conclude, and we concur, that blood transfusion therapy presents the special hazard of isoimmunization, possibly on first exposure, and with increasing likelihood on subsequent exposures. They emphasize the need for increased accuracy in crossmatching and for more efficient use of specific components (33-35).

SUMMARY

The status of military and civilian blood banking is discussed from several viewpoints. The progress made in collecting, processing, storage, and clinical use is reviewed from 1940 to 1972. Several major obstacles have been either eliminated or substantially improved. Blood component therapy has changed the overall operation of blood banks in a remarkable manner. It has now made possible the use of multiple management techniques, heretofore not possible, due to the short shelf life of whole blood. These management techniques include: economics, accounting, mathematics and statistics, and the behavioral sciences. The gross waste of red blood cells tolerated until recently is no longer existent; nor is the commercial plasma salvage technique the only outlet for whole blood not used for patients. The transition to the newer operation of blood component preparation has not come too soon. Our blood donor base, which represents a critical national resource in peace and war, is being eroded by drug abuse, hepatitis carriers, sickle cell trait and disease, malaria, and immunizations. This is coupled with donor apathy, rising cost of blood bank operations, and the problems inherent in recruiting, training, and retaining qualified blood bank personnel.

Procedures are provided for the preparation of several components followed by a discussion of their usefulness and precautions.

In conclusion, we must recognize that with more efficient production, testing, and use of specific components, implementation of sound management principles, increased training programs, and continuing research and development--proper conservation of our blood resources can be achieved. Expansion of frozen blood banking undoubtedly will make a significant contribution toward this goal.

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A N N E X L
ANTISERA EVALUATION
AND
OTHER CONSULTATION SERVICES

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ANTISERA EVALUATION
AND
OTHER CONSULTATION SERVICES

BROCHURE

by

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Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

June 1972

Approved for public release; distribution unlimited.

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MISSION STATEMENTS

Defense Medical Materiel Board: Established by the Secretary of Defense to provide coordination, advice, and assistance on the professional/technical aspects of medical materiel and in the field of medical supply.



Captain R. F. C. MacPherson,
MC, USN, Director, Defense
Medical Materiel Board



Mrs. Elise N. Hayes,
Staff Member, Defense
Medical Materiel Board

Defense Personnel Support Center Medical Mission: Procures, stores, stocks, and issues items of medical materiel standardized by the Defense Medical Materiel Board, based on the logistic requirements of the individual medical services.

SUPPORT AGREEMENT

1. The US Army Medical Research Laboratory (USAMRL) agrees to provide services upon written request from the Directorate of Medical Materiel, Defense Personnel Support Center (DPSC), on the following types of items supplied by the receiving activity:

- a. Plasma protein fraction.
- b. Blood grouping sera.
- c. Bromelin, ficin, papain, and trypsin enzyme solutions.
- d. Serum, antihuman, Coombs test.
- e. Dextran.
- f. Albumin normal human serum.
- g. Albumin serum reagent, bovine.
- h. Globulin, tetanus immune.
- i. Globulin, immune serum.
- j. Globulin, Rh₀ immune.
- k. Other blood derivatives and related products.
- l. Pyrogen testing.
- m. Blood bags.

2. USAMRL will test other blood related equipment and supplies not described above upon mutual agreement with DPSC.

3. USAMRL will conduct workshop courses (duration: 5 days) for medical materiel inspectors of DPSC.

4. Upon completion of any examination, USAMRL will notify DPSC of any evidence of noncompliance with specifications and/or unsuitability for issue and use.

Agreement number Z2-A2250F-0001-2, dated 20 September 1971.

ACKNOWLEDGMENTS

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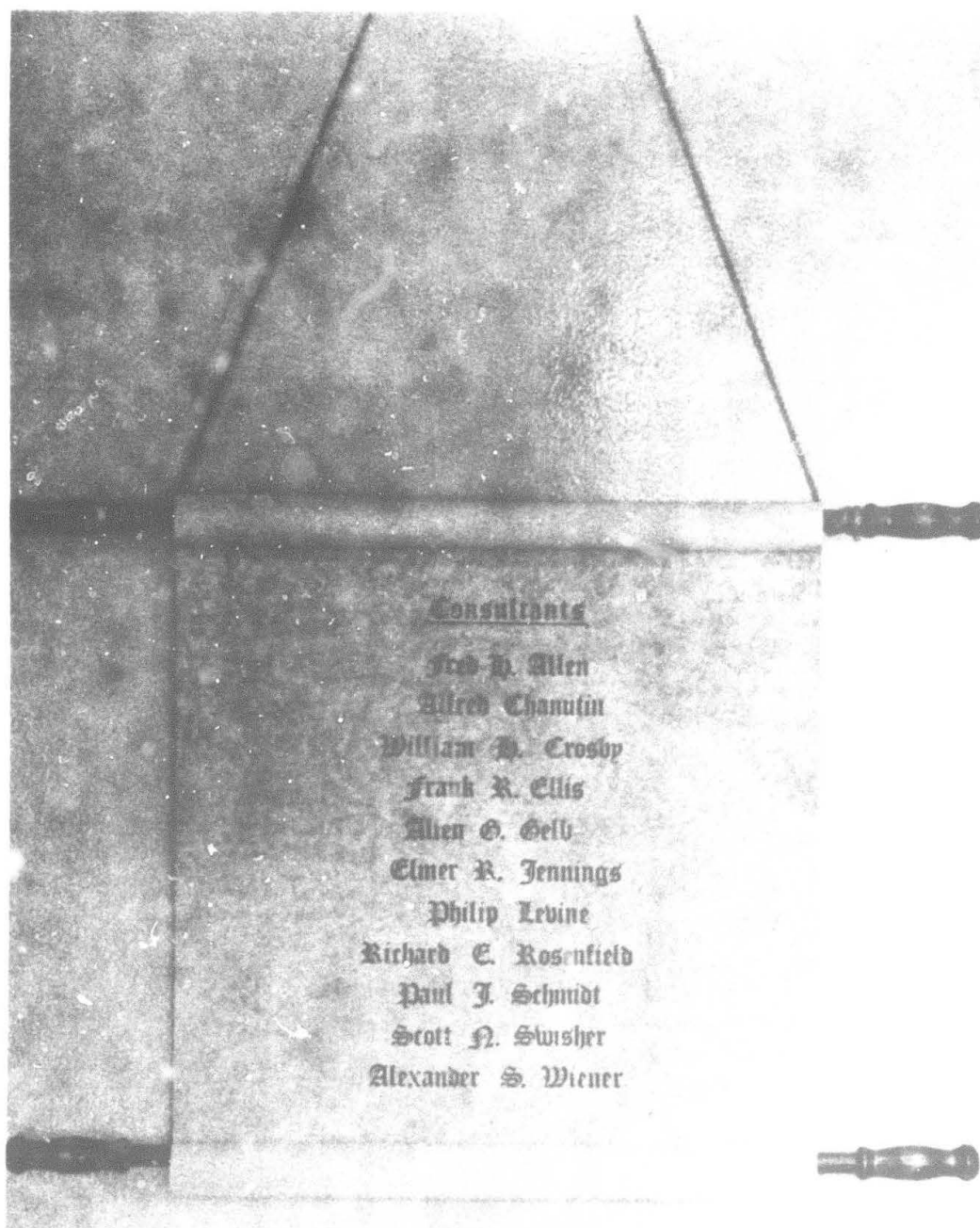


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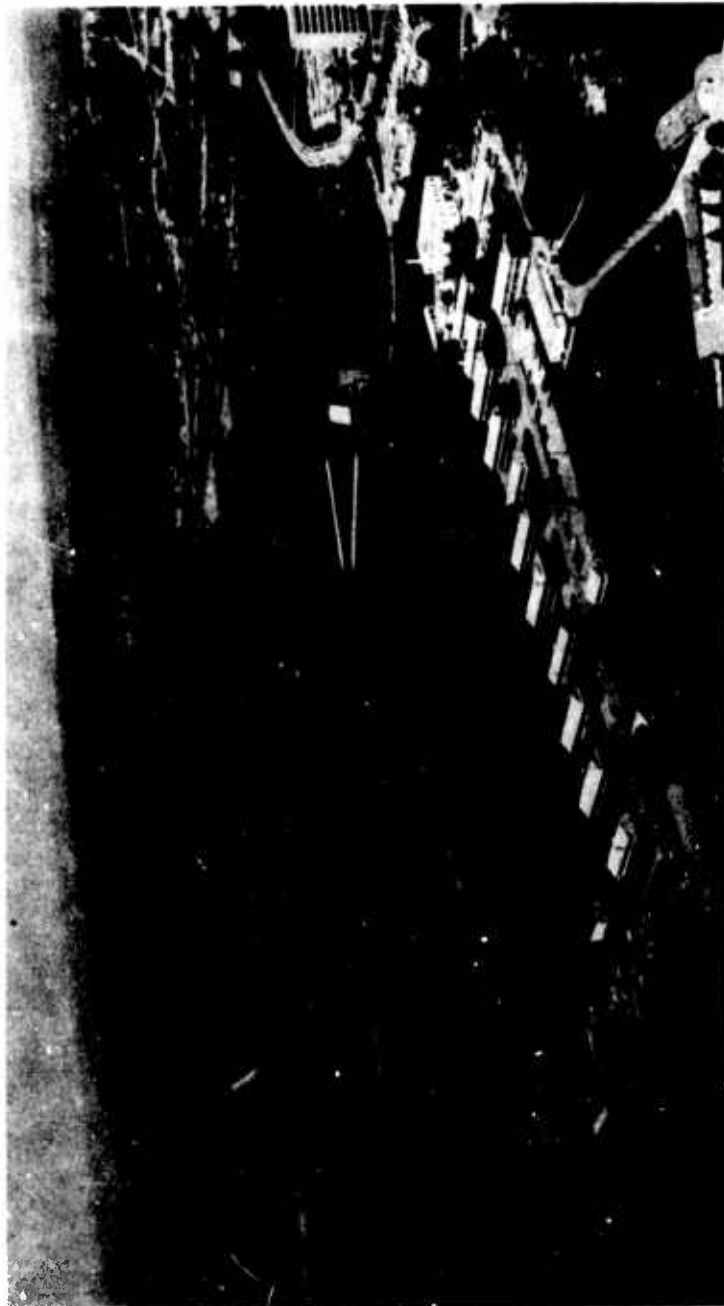


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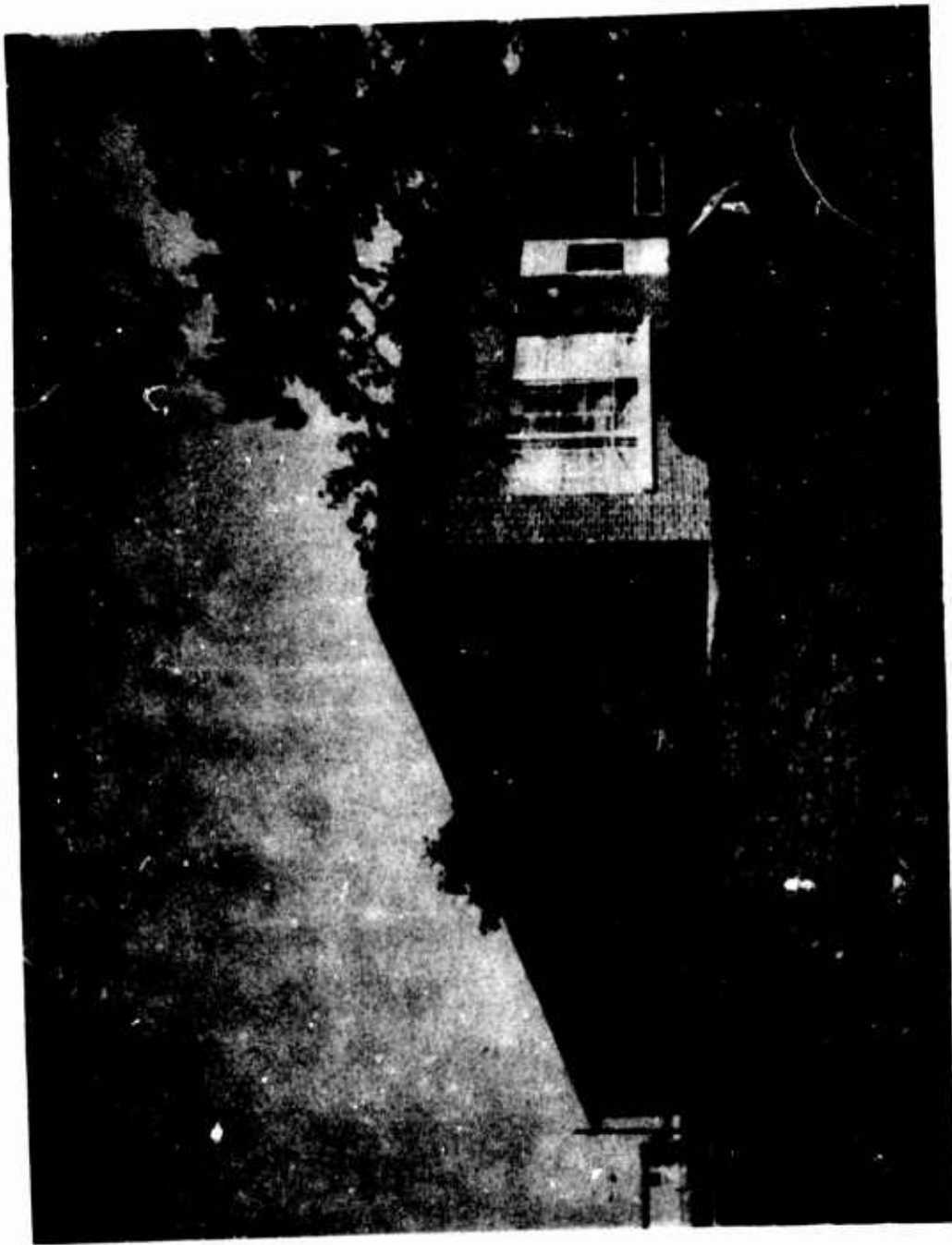
CONSULTANTS' SCROLL



AERIAL VIEW OF USAMRL



BUILDING 1006 - BLOOD BANK CENTER



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PROCESSING LABORATORY

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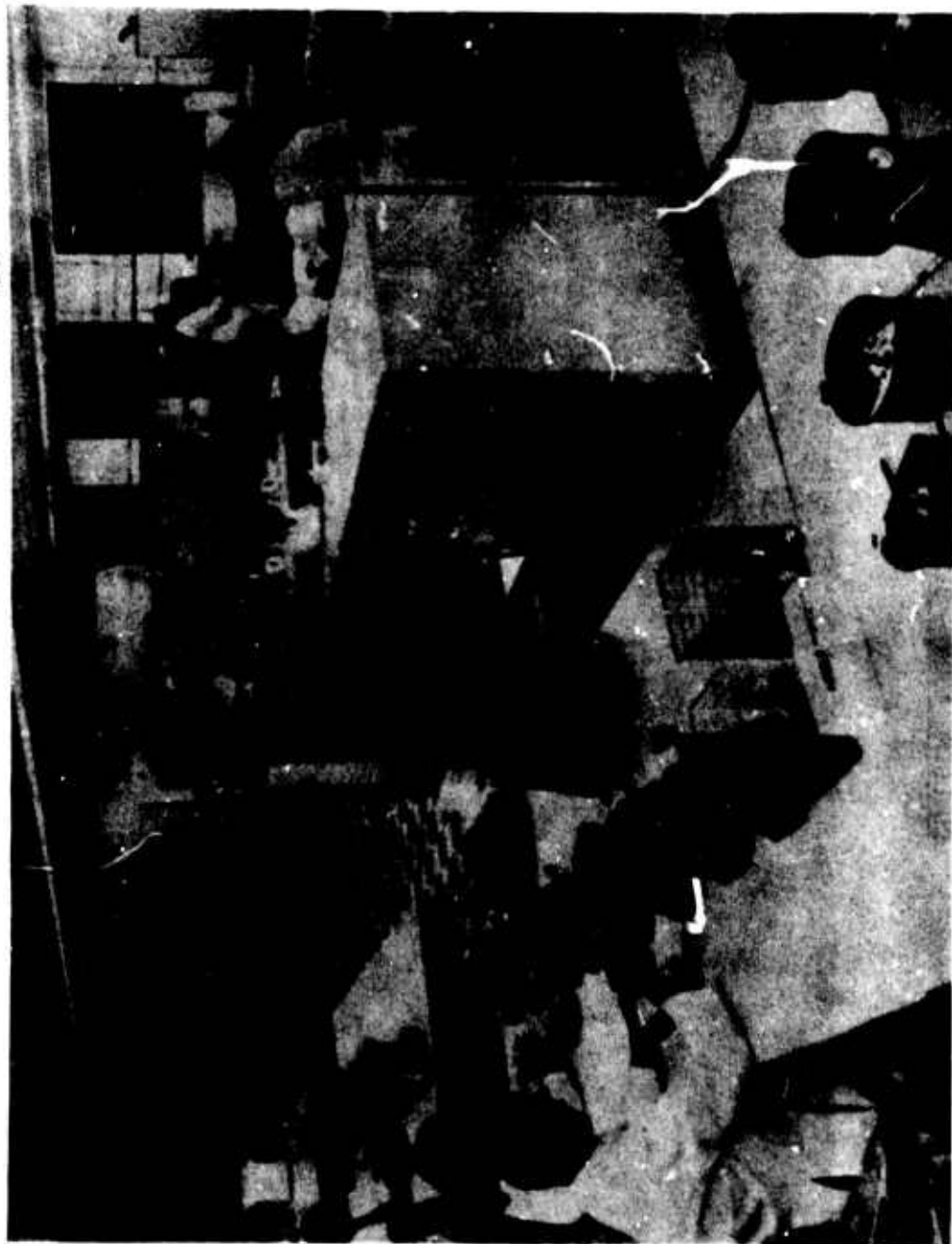


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INTRODUCTION

The Blood Bank Center (BBC), US Army Medical Research Laboratory (USAMRL), Fort Knox, Kentucky, operates a reference laboratory. One important function is to evaluate each lot of blood bank antisera purchased on government contract.* The criteria for evaluation are developed from the performance requirements (essential characteristics) which are established by the Defense Medical Materiel Board (DMMB) and incorporated in the purchase description by the Defense Personnel Support Center (DPSC).

Before any laboratory evaluation, a blood bank reagent must conform to the existing minimum requirements established by the Division of Biologics Standards (DBS), National Institutes of Health (NIH). A copy of the NIH release form must accompany the material submitted. Reference standard reagents from the Division of Biologics Standards, National Institutes of Health, are tested in parallel with all blood group reagents submitted to the Blood Bank Center Reference Laboratory for evaluation.

A contract for a particular antiserum is awarded by the Defense Personnel Support Center (DPSC) after the reference laboratory certifies that the antiserum conforms to DPSC specifications. During bottling of any lot, a quality assurance representative from DPSC is present; at this time 12 bottles are selected at random and shipped directly to the BBC Reference Laboratory by him. Six of these samples are tested before shipment is released; the remaining six are stored as reference samples at the laboratory.

*The following products currently under contract are tested:

1. Anti-A, liquid 5 ml, 6505-159-8475.
2. Anti-A, dried, equivalent to 5 ml, 6505-975-0614.
3. Anti-B, liquid, 5 ml, 6505-159-8500.
4. Anti-B, dried, equivalent to 5 ml, 6505-975-0615.
5. Anti-A,B, dried, equivalent to 5 ml, 6505-935-3998.
6. Anti-A,B, liquid, 5 ml, 6505-584-3038.
7. Anti-Rh₀, liquid, 5 ml, 6505-159-8575.
8. Anti-Rh₀, dried, equivalent to 5 ml, 6505-975-0613.
9. Anti-Rh₀rh'rh", liquid, 5 ml, 6505-684-8664.
10. Antihuman, 2 ml, 6505-071-0611.
11. Antihuman, 10 ml, 6505-065-0024.
12. Albumin, bovine, 22%, 10 ml, 6505-890-1639.

The procedures used in testing antiserum are detailed in Annexes A-G.

The DMMB performance requirements for titer, avidity, and specificity for ABO and Rh sera follow:

TABLE 1
ABO Grouping Sera

Cells	Anti-A			Anti-B			Anti-A,B		
	Titer	Avidity Begin- ning	Com- plete	Titer	Avidity Begin- ning	Com- plete	Titer	Avidity Begin- ning	Com- plete
A ₁	512*	5"	30"				512*	5"	30"
A ₂	128	5"	30"				128	5"	30"
A ₁ B	256	5"	30"	256	5"	30"	256	5"	30"
A ₂ B	64	45"	3'	256	5"	30"	128	5"	30"
B				512*	5"	30"	512*	5"	30"

*1:256, June 1972.

Antibodies must react with the corresponding antigens only. This is tested by using both serum and saline suspensions of group A, B, O, and AB bloods. The tube and slide methods are used in testing at least ten A's, ten B's, ten O's, and five AB's. A 4+ reaction after centrifugation is required with the tube method.

Tests are performed to insure that no hemolysins and/or nonspecific immune antibodies are present.

TABLE 2
Rh Typing Sera

Cells	Anti-Rh ₀			Anti-Rh ₀ rh'rh"		
	Titer	Avidity Beginning	Complete	Titer	Avidity Beginning	Complete
R ₁ R ₁	64	15"	2'			
R ₂ R ₂	64	15"	2'			
R ₁ R ₂	64	15"	2'			
R ₁ r	64	15"	2'			
R ₀ r				64	15"	2'
r'r				4	30"	2'
r"r				4	30"	2'

Antibodies must react with the corresponding antigens only and are tested with serum suspended cells by the tube and slide methods. In the test tube method at room temperature, the degree of positive agglutination reaction with Rh₀ cells must be ++++ when used according to the directions of the manufacturer. No incubation is permitted. Reading of reaction must be made immediately after spin. At least ten random positive bloods and five negative bloods are used in testing. The anti-Rh₀ must be suitable for testing for the Rh₀ variant Rh₀ by the indirect Coombs method.

Some of the more important DMMB performance requirements for anti-human sera and bovine albumin follow:

Antihuman Serum

Antihuman serum is tested using the block Coombs titration. The antihuman serum, when diluted 1:16 in saline, must cause agglutination of sensitized Rh₀ positive erythrocytes producing a 1+ reaction, one serial dilution higher than the basic titer of the anti-Rh₀ serum. It must be capable of detecting antibodies in the Rh, Duffy, Kell, Lewis, and Kidd systems and of detecting immune anti-A and immune anti-B in serum by the indirect antiglobulin method.

Using the direct antiglobulin method, the antiserum must detect coated cells from an acquired hemolytic anemia, as well as coated cord cells in cases of mother-child ABO incompatibility. It must detect both gamma and nongamma immunoglobulins.

Specificity of reagent must permit microscopic examination in blood transfusion compatibility testing.

Albumin, Bovine 22%

Albumin, bovine, must be a concentrated 22% ($\pm 2\%$) solution suitable for use in Rh testing, Rh antibody titrations, and compatibility testing. The pH must be between 7.0 and 8.0; the sodium chloride content between 700 and 1,000 mg/100 ml. The albumin solution must not cause hemolysis, crenation, or rouleaux formation of red blood cells.

In addition to testing blood bank reagents the BBC Reference Laboratory offers the following consultation services to any military installation.

CONSULTATION SERVICES AVAILABLE AT USAMRL

1. Immunohematological studies.
 - a. Antibody detection and identification.
 - b. Crossmatch problem assistance.
 - c. Transfusion reaction studies and assistance.
 - d. Screening for rare donors.
2. Forensic studies.
 - a. ABO determinations.
 - (1) Blood crusts.
 - (2) Blood stains.
 - (3) Seminal stains.
 - (4) Saliva stains.
 - (5) Bone.
 - (6) Hair.
 - (7) Fingernails.
 - b. Precipitin testing.
 - c. Paternity studies.
 - d. Hemoglobin studies.
3. Miscellaneous studies.
 - a. Pyrogen studies.
 - b. Gm testing (referral).
 - c. Special studies upon request.
 - d. Hepatitis (Australia antigen) screening.
 - e. Analysis of blood bank reagents, purchased through DPSC involved in complaints.
 - f. Coagulation studies.

Consultation forms are available upon request. See Annex H for a sample form.

The BBC Reference Laboratory may be reached by telephone:

Day	(502) 624-6656/7051
Night (CQ)	(502) 624-1647
Autovon	464-6656/7051/1647

BLOOD BANK FELLOWS



Blood Bank Fellows (cont)



DPSC WORKSHOP



ANNEX A

PROCEDURE FOR TESTING ANTI-A

1. Titer.

- a. Five rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).
- b. In the first row place 0.7 ml of saline in each of the ten tubes, using a 1 ml pipette.
- c. With a 1 ml pipette, place 0.7 ml of anti-A in the first tube. Discard pipette.
- d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the four tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.
- e. Using a clean pipette, mix and repeat as in d above through the tenth tube.
- f. Place 0.1 ml of a 2% suspension of A₁ cells in the second row, 0.1 ml of a 2% suspension of A₂ cells in the third row, 0.1 ml of a 2% suspension of A₁B cells in the fourth row, and 0.1 ml of a 2% suspension of A₂B cells in the fifth row. Mix. Spin in a serofuge for 30 seconds. Read for agglutination. Let tubes sit at room temperature for 15 minutes and reread.

2. Avidity.

- a. Use a 10% suspension of the same cells used in the titer (A₁, A₂, A₁B, and A₂B).
- b. Place one drop of the cell suspension on a slide and one drop of anti-A. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

- a. Usually ten random group A bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)
- b. Using blood of A₁, A₂, A₁B, A₂B, B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-A are used. Spin immediately. Read for agglutination.

c. These same six blood groups are tested by the stick-tube method giving a serum suspended cell of a 2% suspension in whole blood. Spin immediately. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-A are placed in three tubes. Place one tube at room temperature, one tube at 37 C, and one tube at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Then the three tubes are kept at room temperature for 2 hours. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.

ANNEX B

PROCEDURE FOR TESTING ANTI-B

1. Titer.

a. Four rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row, place 0.7 ml of saline in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.7 ml of anti-B in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the three tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of B₁ cells in the second row, 0.1 ml of a 2% suspension of A₁B cells in the third row, 0.1 ml of a 2% suspension of A₂B cells in the fourth row. Mix. Spin in a serofuge for 30 seconds. Read for agglutination. Set tubes aside for 15 minutes at room temperature and reread.

2. Avidity.

a. Use a 10% suspension of the same cells used in the titer (B, A₁B, and A₂B).

b. Place one drop of the cell suspension on a slide and one drop of anti-B. Mix. Observe for beginning and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random group B bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)

b. Using blood from A₁, A₂, A₁B, A₂B, B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-B are used. Spin immediately. Read for agglutination.

c. These same six blood groups are tested by the stick-tube method, giving a serum suspended cell of a 2% suspension in whole blood. Spin. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-B are placed in three tubes. Place one tube at room temperature, one at 37 C, and one at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Keep the three tubes at room temperature for 2 hours. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.

ANNEX C

PROCEDURE FOR TESTING ANTI-A,B

Cells needed: A₁, A₂, B, A₁B, and A₂B - 2% suspensions in saline.

1. Titer.

a. Six rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.8 ml of saline in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.8 ml of anti-A,B in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the five tubes in the back and 0.8 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of A₁ cells in the second row, 0.1 ml of a 2% suspension of A₂ cells in the third row, 0.1 ml of a 2% suspension of B cells in the fourth row, 0.1 ml of a 2% suspension of A₁B cells in the fifth row, and 0.1 ml of a 2% suspension of A₂B cells in the sixth row. Mix. Spin in serofuge for 30 seconds. Read for agglutination. Let tubes sit at room temperature for 15 minutes and reread without spinning.

2. Avidity.

a. Use a 10% saline suspension of same cells used in the titer (A₁, A₂, B, A₁B, and A₂B).

b. Place one drop of the cell suspension on a slide and one drop of anti-A,B. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually 10-15 random group A, B, and AB bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)

b. Using blood of A₁, A₂, B, A₁B, A₂B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-A,B are used. Spin immediately. Read for agglutination.

c. The same six blood groups are tested by the stick-tube method giving a serum suspended cell of a 2% suspension in whole blood. Spin immediately. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-A,B are placed in three tubes. Place one tube at room temperature, one tube at 37 C, and one tube at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Then set aside the three tubes for 2 hours at room temperature. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.

ANNEX D

PROCEDURE FOR TESTING ANTI-Rh₀

Cells needed: Group O, R₁r, R₁R₁, R₁R₂, R₂R₂ - 2% suspensions in 22% albumin.

1. Titer.

a. Five rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.7 ml of group AB serum in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.7 ml of anti-Rh₀ in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the four tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of R₁r cells in the second row, 0.1 ml of a 2% suspension of R₁R₁ cells in the third row, 0.1 ml of a 2% suspension of R₁R₂ cells in the fourth row, and 0.1 ml of a 2% suspension of R₂R₂ cells in the fifth row. Mix. Incubate at 37 C for 1 hour. Mix. Spin in a serofuge for 45 seconds. Read for agglutination.

2. Avidity.

a. Use as whole blood the same cells used in the titer (R₁r, R₁R₁, R₁R₂, and R₂R₂).

b. Place two drops of the cell suspension on a slide (heated to 37 C), add one drop of anti-Rh₀. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random Rh positive and five Rh negative bloods are tested by the slide and stick-tube methods.

b. Test, by slide and stick-tube methods, using cells of R₁r, R₁R₁, R₁R₂, and R₂R₂ in the appropriate suspensions.

c. Using known Rh₀ positive and negative cells, test for this Rh variant. Place two drops of anti-Rh₀ in a tube, two drops of 22% albumin in a second tube (negative control). Add one drop of a 2% suspension of cells to each tube. Incubate at 37 C for 30 minutes. Wash three times, add two drops of Coombs, spin and read.

4. Clarity. Material should be clear and free of particulate matter.

ANNEX E

PROCEDURE FOR TESTING ANTI-Rh₀rh'rh''

Cells needed: Group 0, R₀r, r'r, r''r - 2% suspension in 22% albumin.

1. Titer.

a. Four rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

b. In the first row place 0.6 ml of group AB serum in each of the ten tubes, using a 1 ml pipette.

c. With a 1 ml pipette, place 0.6 ml of anti-Rh₀rh'rh'' in the first tube. Discard pipette.

d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the three tubes in the back and 0.6 ml in the tube on the right (1:4). Discard pipette.

e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

f. Place 0.1 ml of a 2% suspension of R₀r cells in the second row, 0.1 ml of a 2% suspension of r'r cells in the third row, and 0.1 ml of a 2% suspension of r''r cells in the fourth row. Mix. Incubate at 37 C for 1 hour. Mix. Spin in a serofuge for 45 seconds. Read for agglutination.

2. Avidity.

a. Use as whole blood the same cells used in the titer (R₀r, r'r, and r''r).

b. Place two drops of the cell suspension on a slide (heated to 37 C), add one drop of anti-Rh₀rh'rh''. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

3. Specificity.

a. Usually ten random Rh positive and five Rh negative bloods are tested by the slide and stick-tube methods.

b. Test by slide and stick-tube methods, using cells of R₀r, r'r, and r''r in the appropriate suspensions.

4. Clarity. Material should be clear and free of particulate matter.

ANNEX F

PROCEDURE FOR TESTING ANTIHUMAN SERUM

1. Materials.

a. Red blood cells. For more reactive tests, use homozygous Rh₀ positive cells preferably of genotype R₂R₂. If these are not available use genotype R₁R₁ or R₁R₂. Reasonably fresh cells should be used.

b. Anti-Rh₀ antiserum. Use anti-Rh₀ antiserum which has a minimum titer of 1:32.

2. Quantitative test procedure.

Sensitization of cells with dilutions of anti-Rh₀.

a. Wash cells in normal saline once. After washing, there should be a minimum packed cell volume of 0.8 ml.

b. Make a 2% cell suspension.

c. Place 5.0 ml of the 2% cell suspension into each of six test tubes (use graduated centrifuge tubes, if possible). Label the tubes 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

d. Add more normal saline to each tube and centrifuge. After centrifugation, each tube should have a 0.1 ml packed cell volume. Aspirate all of the saline from each tube.

e. While the cells are being centrifuged, the dilutions of the anti-Rh₀ antiserum can be made. Label six 13 x 100 mm test tubes 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

f. Place 12.0 ml normal saline in tube labeled 1:16, place 6.0 ml normal saline in the remaining tubes.

g. Add 0.8 ml anti-Rh₀ to tube 1:16. Rinse the pipette in the tube several times. This gives a dilution of 1:16 anti-Rh₀ in a total volume of 12.8 ml.

h. Make twofold dilutions in the remaining tubes by removing 6.0 ml from tube 1:16 into tube 1:32, and so on. Discard the remaining 6.0 ml of the 1:512 dilution.

i. Place 4.9 ml of each dilution of anti-Rh₀ into the appropriately labeled tube containing 0.1 ml packed cells. Mix well to resuspend cells.

j. Incubate at 37 C for 1 hour.

k. Wash four times with normal saline. This is important because insufficient washing may cause a false negative reaction.

l. Add 4.9 ml normal saline and resuspend packed cells.

m. Place 0.1 ml of sensitized cells in six tubes from each dilution of anti-Rh₀, a total of 36 tubes. Add 0.1 ml of antihuman (Coombs) serum, using undiluted 1:2, 1:4, 1:8, 1:16, and 1:32 dilutions. (See Diagram #3. Titration of Antihuman Serum, Appendix A, Minimum Requirements: Antihuman Serum for the Antiglobulin Test, NIH.)

n. Centrifuge and read. (See 3.5 potency requirements, Minimum Requirements: Antihuman Serum for the Antiglobulin Test, NIH.)

3. Qualitative test procedure.

a. Place two drops of Rh₀ positive cells (2% cell suspension) in a 12 x 75 mm test tube.

b. Add two drops of 1:16 dilution of anti-Rh₀.

c. Spin and read. (Test should be negative; if positive, make a higher dilution of anti-Rh₀.)

d. Incubate at 37 C for 1 hour.

e. Spin and read. (Test should still be negative.)

f. Wash four times with saline and decant completely after last wash.

g. Add two drops of antihuman serum. Centrifuge and read. (This should be positive.)

4. Potency testing (using a known positive antigen-antibody system):

a. Depending on the titer of the antisera used, make either a 1:10 or a 1:20 dilution of anti-rh', anti-rh'', anti-hr', and anti-hr''.

b. Place two drops of each dilution into a test tube.

c. Add two drops of a 2% cell suspension of the corresponding Rh antigen.

d. Incubate at 37 C for 30 minutes.

e. Wash four times with normal saline and decant completely after last wash.

- f. Add antihuman serum, spin, and read. (Tests should be positive.)
- g. Repeat, using undiluted anti-K, anti-Jk^a, anti-Le^a, and anti-Fy^a antisera with two drops of a 2% cell suspension of their corresponding antigens. (Tests should be positive.)
- h. Test for immune anti-A and anti-B, using group O serum (previously known to have immune A and B) according to the AABO screening method. (See pages 59 and 60 of AABO Manual, 5th edition.)

BLOCK COOMBS TITRATION

Coombs Dilution	Dilution of Anti-Rh ₀ Sensitized Cells (Group O)					
	1:16	1:32	1:64	1:128	1:256	1:512
Undiluted						
1:2						
1:4						
1:8						
1:16						
1:32						

<u>Dilution for Coombs</u>	<u>Amount of Saline</u>	<u>Amount of Coombs</u>
1:2	0.4 ml	0.4 ml
1:4	0.6 ml	0.2 ml
1:8	0.7 ml	0.1 ml
1:16	0.75 ml	0.05 ml
1:32	1.55 ml	0.05 ml

5. Anticomplement activity (C'4 and C'3).

a. Materials.

- (1) Five percent dextrose in water.
- (2) Isotonic saline.
- (3) Fresh clotted blood (less than 24 hours old), clot and serum separated.
- (4) Liquid K₂ EDTA, 5 mg per drop.
- (5) Parafilm.
- (5) Five Pasteur pipettes.

b. Procedure.

- (1) Mark three 13 x 100 mm tubes at the 2 ml level and number them 1, 2, and 3.
- (2) Transfer 5% dextrose in water to tubes 1 and 2 and fill to the 2 ml level.
- (3) Transfer isotonic saline to tube 3 and fill to 2 ml level.
- (4) Add three drops of liquid EDTA to tube 2.
- (5) Add five drops of fresh serum to each tube.
- (6) Cover tubes with parafilm and mix several times.
- (7) Add three drops of fresh whole clotted blood (from the same donor as the serum) to each tube.
- (8) Cover tubes with parafilm and mix well.
- (9) Incubate all three tubes for 10 minutes at 37 C.
- (10) Label three 10 x 75 mm tubes 1, 2, and 3.
- (11) Place two drops of the mixed cell suspensions from the larger tubes into the appropriately numbered small tubes.
- (12) Wash the cells three times in saline.
- (13) Add one drop antiglobulin reagent, serofuge, and read.

RECORD RESULTS

Cell	Low Ionic Strength		Normal Ionic Strength
	1 Complement Coated	2 Complement Blocked	3 No Coating
Antiglobulin Test			

ANNEX G

PROCEDURE FOR TESTING BOVINE ALBUMIN

Cells needed: Group O, R₁r, R₁R₁, R₁R₂, R₂R₂.

1. Test for hemolysis, crenation, and rouleaux formation of red blood cells.

- a. Place two drops of albumin into several tubes.
- b. Add two drops of a 2% suspension of group O cells to each tube.
- c. Observe macroscopically and microscopically for crenation of cells, hemolysis, and rouleaux formation. None should be present.

2. Test for clot formation in crossmatching procedure.

- a. Place two drops of plasma in tube.
- b. Add one drop of a 2% suspension of cells (obtained from segment on bag).
- c. Add two drops of albumin.
- d. Incubate at 37 C for 30 minutes, spin, and read. Observe closely for clot formation. Test should be negative.

3. Test for observing hemolysis.

- a. Place 0.1 ml of serum (known to have a hemolysin) in a tube.
- b. Add 0.1 ml of a 2% suspension of A₁ or B cells.
- c. Add 0.1 ml of albumin.
- d. Incubate at 37 C for 1 hour.
- e. Spin and read. Hemolysin should be present.

4. Quantitative testing.

- a. Make serial dilutions of a previously tested anti-Rh₀ in group AB serum.
- b. Make 2% cell suspensions of group O, R₁r, R₁R₁, R₁R₂, and R₂R₂ cells in the albumin.

c. Add 0.1 ml of the cell suspension to 0.1 ml of the anti-Rh₀ dilution.

d. Incubate at 37 C for 1 hour.

e. Mix. Spin for 45 seconds and read. Titer should be the same as when previously tested.

5. Sodium chloride content.

a. Determine the chloride content.

b. Sodium chloride content may then be determined by using this formula:

$$\text{mEq Cl/l} \times 5.85 = \text{mg NaCl/100 ml}$$

c. Should be between 700-1000 mg/100 ml.

6. pH determination. pH of the albumin should be between 7.0 and 8.0.

7. Percent of albumin. Albumin content should be 22% \pm 2%.

ANNEX H

Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

REQUEST FOR CONSULTATION

Send Report To: _____ Date: _____

Name: _____

Hospital: _____

Street: _____

City & State: _____ Zip Code: _____

Telephone No: _____ Area Code: _____

Send specimen to: Reference Laboratory
Blood Bank Center
US Army Medical Research Laboratory
Fort Knox, Kentucky 40121

Procedure for submitting samples:

1. Send freshly drawn samples, clearly labeled with full name and date.
2. Send 15 to 20 ml clotted blood and 5 ml anticoagulated blood. SEPARATE MOST OF SERUM FROM CLOT
3. Send specimens AIR MAIL, SPECIAL DELIVERY, and label "Blood specimens - refrigerate as soon as possible." Mail container to arrive at Reference Laboratory between Monday and Friday, if possible.
4. Notify the Reference Laboratory by telephone of the shipment.
Autovon: 464-6656
Commercial: 624-6656, Area Code 502

INFORMATION CONCERNING CASE

1. Patient's name _____ Serial No. _____

Sex _____ Age _____ Race _____

Diagnosis _____

2. ALL FEMALE PATIENTS:

Number of pregnancies _____ Any difficulty? _____

Number of exchanges, if necessary _____

3. ALL PATIENTS:

Number of transfusions and dates _____

Number of group O (universal donor) units received _____

Type of reaction and number of units received _____

Estimate number of units needed _____

4. DIFFICULTY ENCOUNTERED:

A. Crossmatch problem _____

1. Saline _____ 2. Albumin _____

3. Coombs _____ 4. Enzyme _____

No. of donors compatible _____ No. of donors incompatible _____

Is patient receiving any drugs? _____ List drugs _____

B. Antibody identification _____

1. Saline _____ 2. Albumin _____

3. Coombs _____ 4. Enzyme _____

C. Hemolytic Disease of the Newborn _____

D. Other (explain in detail) _____

ANNEX I

AVAILABLE SCIENTIFIC LITERATURE

US ARMY MEDICAL RESEARCH LABORATORY REPORTS

<u>Report Number</u>	<u>Title</u>
671	Blood Components - Their Preparation and Use
677	Interaction of Progesterone and Aldosterone With Red Blood Cells of the Rat
678	Military Blood Banking - Identification of the Group O Universal Donor for Transfusion of A, B, and AB Recipients - An Enigma of Two Decades
707	Screening Procedures Employing Semiautomated and Fully Automated Technics
708	Effect of Immunizations on Blood Group Antibody Production
717	Inhibitory Properties of Serum Proteins on the Enzymatic Sequence Leading to Lysis of Red Blood Cells by Snake Venom
719	Comparison Studies of Whole Blood Stored in ACD and CPD and With Adenine
735	The Effect of Sulfhydryl Reagents on the Binding of Human Hemoglobin to Haptoglobin
739	Testing of Blood Grouping Cards Under Field-Type Conditions
741	Survival of ACD Blood After Serial Storage
747	The Glomerular Filtration of Hemoglobin: A Proposed Mechanism
752	Rate of Hemolysis of Fresh and Stored Human Red Blood Cells and the Effect of Progesterone
755	Clinical Hematological Values, Erythrocytic Indices and Osmograms of <i>Cercopithecus torquatus atys</i> and <i>Papio anubis</i>
756	A Continuous Body Temperature Monitoring System for Utilization in Pyrogen Testing
758	Stored Whole Blood after Long Distance Transportation

<u>Report Number</u>	<u>Title</u>
762	Investigation of Materials and Methods for Air Delivery of Whole Blood and Blood Products
777	Subunit Dissociation of Certain Abnormal Human Hemoglobins
781	Long-term Preservation of Biologicals for the Forensic Laboratory and Their Areas of Application
785	An Adaptation of the Peters and Van Slyke Method for Measuring Whole Blood Oxygen Dissociation Equilibria
790	Hemoglobin Function in Stored Blood
792	Studies on Stored Liquid Whole Blood. II. Use of Packed Red Cells
794	<i>In Vivo</i> and <i>In Vitro</i> Studies on BME Hemoglobin
798	Demonstration of Blood Group Substance A Bound to <i>Pasteurella pestis</i>
799	Preservation of Human Blood from Male and Female Donors
803	Automated Detection of Gm Factors of Human γ G Globulin
804	Human Serum Antiglobulins and Immunization
805	Evaluation of Automated Multichannel Blood Grouping Apparatus. II. Comparison with Manual and Dried Reagent Methods
806	Evaluation of Automated Multichannel Blood Grouping Apparatus. I. Procedure and Reagent Standardization for Blood Grouping
807	The Occurrence of Blood Group Substances A and B in Proprietary Gamma Globulin of Placental Origin
808	Clinical Evaluation of Transfused Blood after Long-term Storage in ACD with Adenine
809	Effects of Environmental Temperature on Selected Blood Shipping Containers
810	Comparison of Autologous and Nonautologous Transfusions of ACD-Adenine Blood

<u>Report Number</u>	<u>Title</u>
815	Serum Agglutinators Reacting with Pepsin Treated Gamma Globulin: I. "Naturally Occurring" Reactants in the Serum of Subhuman Primates
816	Study of Military Blood Banking and Crossmatching Using Blood Group Antigens Stored over Five Months in ACD-Adenine
818	ABO Antibodies. I. Methods for Quantification of ABO Hemoly- sins and Soluble Blood Group Substances A and B
826	Studies on Stored Liquid Whole Blood. I. Effect of Volume Transfused on <i>In Vivo</i> Survival Measurement
828	Evaluation of Automated Multichannel Blood Grouping Apparatus. III. Studies on Detection of Human Hemagglutinins
830	Evaluation of an Automated Method for Blood Grouping in the Military Service - A System Analysis
833	Studies on Stored Liquid Whole Blood. III. Evaluation of Plastic Collection Containers
834	A Semiautomated Method for Quantitative Fibrinogen Determina- tions
836	The Hemoglobin Function of Blood Stored at 4°C
837	The Therapy of Experimental Hemorrhagic Shock with Red Cell Stroma Free Hemoglobin Solution
838	Evaluation of Stroma Free Hemoglobin Solution: Effects on Renal Function in Cynomolgus Monkeys
839	The Effect of Methylene Blue Addition to Whole Blood During Prolonged Storage
840	Relative Viscosity and Specific Gravity of Human Blood During Cold Storage
842	Blood Shipping Boxes Evaluated Under Varying Modes of Heat Exposure
843	Isolation and Initial Studies on a Proteinase from Human Erythrocyte Membranes
844	Changes in Human Erythrocyte Membrane Proteins During Storage

Report
Number

Title

- 845 Investigation of Nephrotoxic Effects of Adenine and Its Metabolic Product, 2,8-Dioxyadenine, on Primates (*Macaca irus*)
- 851 The Role of Automated Blood Grouping as an Information Retrieval System
- 852 A Single Card Laboratory Reference Index System
- 854 A Consideration of Some Correlates of Fainting in Blood Donors
- 855 A Comparative Study of the Incidence of Blood Donor "Reactors" in Smokers and Nonsmokers
- 856 The Control of Hemoglobin Function in Blood Stored for Transfusion Purposes
- 858 A Fail-Safe Approach to Incompatible Blood Transfusions
- 863 A Biphasic Extraction for 2,8-Dioxyadenine
- 865 A Quality Control Approach to Improved Donor Care
- 867 Standardization of Blood Transfusion Reaction Studies in the Military. Delegation of Responsibility for a Medical Team Concept. Role of the Hospital Transfusion Board
- 868 Evaluation of Human Plasma After Prolonged Storage in Plastic Containers
- 870 The Clotting System of Monkeys: A Comparison of Coagulation Factors and Tests between Cynomolgus Monkeys (*Macaca irus*) and Humans
- 875 Biochemical and Physical Properties of Stored Male and Female Donor Blood
- 876 Effect of Plasma Removal on Blood Stored in ACD With Adenine
- 877 The Hemoglobin Function and 2,3-DPG Levels of Blood Stored at 4°C in ACD and CPD: The pH Effect
- 878 Hemoglobin Function in Stored Blood: IV. Red Cell Viability and Function in ACD and CPD With Adenine and Inosine
- 880 Tissue Transplantation - The Universal Donor and Blood Group Antibodies

<u>Report Number</u>	<u>Title</u>
881	Biological Alterations Occurring During Red Cell Preservation
882	Studies on Stored Liquid Whole Blood. IV. Effects of Temperature and Mechanical Agitation
887	Effect of Heparinized Saline Infusion and Hypotension on Calcium Homeostasis in the Dog
891	Saliva Agglutinins and Automated Methods for Universal Donor Screening
892	A Practical Synopsis of Consumption Coagulopathy
893	The Murayama Test. Part I: Evidence for the Modified Murayama Hypothesis for the Molecular Mechanism of Sickling
894	The Murayama Test. Part II: Principles, Technique, Interpretation, and Data
895	Sickling Reversed and Blocked by Urea in Invert Sugar: Optical and Electron Microscopy Evidence
896	Sickle Cell Crisis Terminated by Use of Urea in Invert Sugar in Two Cases
897	Modified Sickledex Tube Test: A Specific Test for S Hemoglobin
898	An Automated Screening Method for the Specific Detection of Homozygous and Heterozygous S Hemoglobin
900	Hemolysis and Intravascular Coagulation Due to Incompatible Red Cell Transfusion in Isoimmunized Monkeys
902	Disseminated Intravascular Coagulation and Renal Failure: Production in the Monkey With Autologous Red Cell Stroma
909	Steroid Hormones in the Preservation of Human Blood
910	Embryonic, Fetal, and Neonatal Hemoglobin Synthesis: Relationship to Abortion and Thalassemia
912	Consumption Coagulopathy: Practical Principles of Diagnosis and Management
914	The Function of Human Hemoglobin: Salt Effects

<u>Report Number</u>	<u>Title</u>
915	Hemoglobin Function in Stored Blood: VI. The Effect of Phosphate on Red Cell ATP and 2,3-DPG
916	Platelet Contamination of Erythrocyte Membrane Preparations
918	Cargo Coding Developments in Military Blood Bank Logistics
924	Hemoglobin Function in Stored Blood: Effects of Salts and Glutathione
925	Hemoglobin Function in Stored Blood: Further Effects of Phosphate on Red Cell ATP and 2,3-DPG
926	Blood Preservation Solutions: A Review
927	The Salivary Anti-A and Anti-B Isoantibody System in Group O Males
929	Plasma Transfusion Reactions in Isoimmunized Monkeys
930	Turbidity Measurements of Solubilized Human Erythrocyte Membranes
931	Military Blood Banking (Civil Disasters)
932	Hemoglobin Function in Stored Blood: IX. A Modified Preservative with Optimal pH to Maintain Red Cell 2,3-DPG (Function) and ATP (Viability)
933	Forensic Aspects of Transfusion Reactions
934	Comparison of Blood Collected by Vacuum Methods and Gravity Flow
935	Changes in Erythrocyte Membranes during Cold Storage II
936	The Detection of Sickle Cell Disease in Large Human Populations by an Automated Technique
937	The Forensic Testing Laboratory, 1971--Problems, Progress, and People
938	Physicochemical Changes in Erythrocyte Membranes During Cold Storage in the Presence of Progesterone
939	Effect of Varying Concentrations of Adenine, Inosine, and Methylene Blue on the Useful Storage Life of Blood

**Report
Number**

Title

- 942 Dithionite Tube Test - A Rapid, Inexpensive Technique for the Detection of Hemoglobin S and Non-S Sickling Hemoglobin
- 943 Automated Dithionite Test for the Rapid, Inexpensive Detection of Hemoglobin S and Non-S Sickling Hemoglobinopathies
- 944 The Murayama Test for Hemoglobin S (A Simplification in Technique)
- 945 Sickledex Test for S Hemoglobin: A Critique
- 955 Automated Quantitation of A and B Blood Group Substances
- 958 Blood Component Logistics
- 960 Hemolytic, Coagulant, and Renal Effects of Transfused IgG and IgM Derived from Plasma of Isoimmunized Monkeys
- 961 Ability of Rabbit IgG Fab' Fragment Specific for a Human Species Antigen to Block Reactivity of HL-A Antisera
- 962 Specificity of a Rabbit Antihuman Lymphocyte Serum
- 963 Passive Suppression Characteristics of a Rabbit Antihuman Lymphocyte Serum
- 964 Management in Military Blood Banking for Conservation of Blood Resources: New Aspects Concerning the Blood Donor Base
- 965 Mass Screening of Military Populations for Hemoglobin S by the Automated Dithionite Test
- 966 A Collected Bibliography of Clinical Advances in Sickle Cell Disease Based on the Murayama Molecular Hypothesis
- 969 Pulmonary Hemorrhage Syndrome as a Manifestation of Disseminated Intravascular Coagulation: Analysis of 10 Cases
- 972 Thermal Destruction of Anti-A₁ and Anti-A(A₂) from Group O and Group B Serum
- 973 Electrocardiographic and Respiratory Changes Observed in Blood Donors During Phlebotomy
- 974 Hemoglobin Function in Stored Blood: XII. Effects of Varying Phosphate Concentrations on Red Cell ATP and 2,3-DPG with Adenine and Inosine

Report
Number

Title

- 975 Blood Preservation Solutions. XI: Raising the pH to Improve Hemoglobin Function
- 978 Urea, Urease, Cyanate, and the Sickling of Hemoglobin S
- 979 The Effects of Platelets on the Storage Properties of Human Erythrocytes
- 980 Sickle Cell Disease: Clinical Advances by the Murayama Molecular Hypothesis

- Monograph Pitfalls of Blood Grouping and Pretransfusion Tests
 (Library of Congress Catalog Card Number 75-606639)
- Monograph Genetics for the Reference and Forensic Testing Laboratory
 (Library of Congress Catalog Card Number 77-175026)
- Monograph Military Blood Banking 1941-1971. Lessons Learned Applicable to Civil Disasters and Other Considerations
 (Library of Congress Catalog Card Number 78-184862)
- Monograph Immunohematology
 (Library of Congress Catalog Card Number 77-175027)
- Monograph Blood Group Immunology: Translation and Reproduction of Early Scientific Treatises
 (Library of Congress Catalog Card Number 76-188448)
- Brochure Antisera Evaluation and Other Consultation Services Available at The Blood Bank Center Reference Laboratory

Translation Series

- Gammelgaard, Arne. On Rare, Weak A Antigens (A₃, A₄, A₅, and A_x) in Man
(Library of Congress Catalog Card Number 64-65449)
- Hartman, Grethe. Group Antigens in Human Organs
(Library of Congress Catalog Card Number 71-606638)
- Selected Contributions to the Literature of Blood Groups and Immunology:
(Library of Congress Catalog Card Number 71-606638)
- Volume I. The ABO System (Dunsford Memorial)

- Volume II. Secretion of Blood Group Substances and Lewis System
- Volume III. Part I. Constitutional Serology and Blood Group Research
Part II. M, N, and P Systems
- Volume IV. Part I. Anthropologic Data
Part II. Blood Groups and Their Areas of Application
- Volume V. Landsteiner Centennial

ANNEX J

MISCELLANEOUS PHOTOGRAPHS

**ACTIVATION AND GROWTH OF BLOOD PROGRAMS
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121**

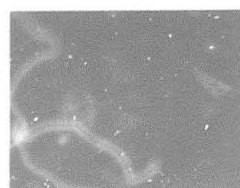
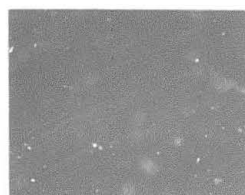
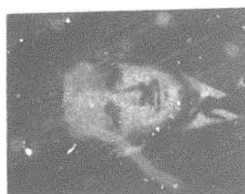
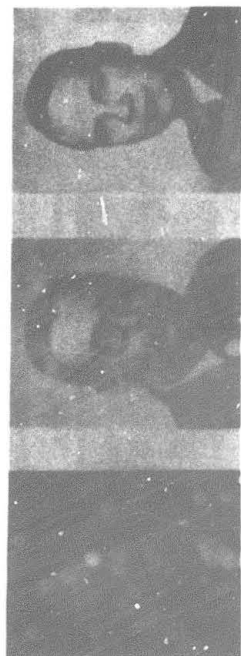
*1964	Staff Study
1965	Blood Transfusion Research Division
1965	Blood Group Reference Laboratory
**1965	Quality Control Monitoring (DPSC)
1965	Blood Bank Fellowship Program (3 Fellows) Army
1966	Medical Corps Officer Training Program
1966	Reference and Forensic Testing Laboratory
1966	Blood Transfusion Division
1967	Institutional Membership, AABB
***1967	Approved Institution of Training AABB-ASCP
1969	Blood Coagulation Laboratory
1969	Transfusion Reaction Model
1969	Blood Components Center
1969	Blood Bank Fellowship (4 Fellows) 3 Army, 1 Navy
1970	Histocompatibility (Lymphocyte Typing) Laboratory
1970	Field Testing Laboratory
1970	311-F1 Blood Bank Training for Enlisted Personnel
1971	Blood Bank Center
1971	Blood Research Division
1971	AABB Reference Laboratory
1971	Blood Bank Fellowship (5 Fellows) 3 Army, 1 Navy, 1 Air Force

FUTURE GOALS

**Frozen Red Blood Cell Bank
Rare Donor Registry**

-
- *Crosby & Camp
 - **Defense Personnel Support Center
 - American Association of Blood Banks
 - ***American Society of Clinical Pathology

US ARMY
 BLOOD TRANSFUSION RESEARCH STAFF 1965-1971
 FORT KNOX, KENTUCKY

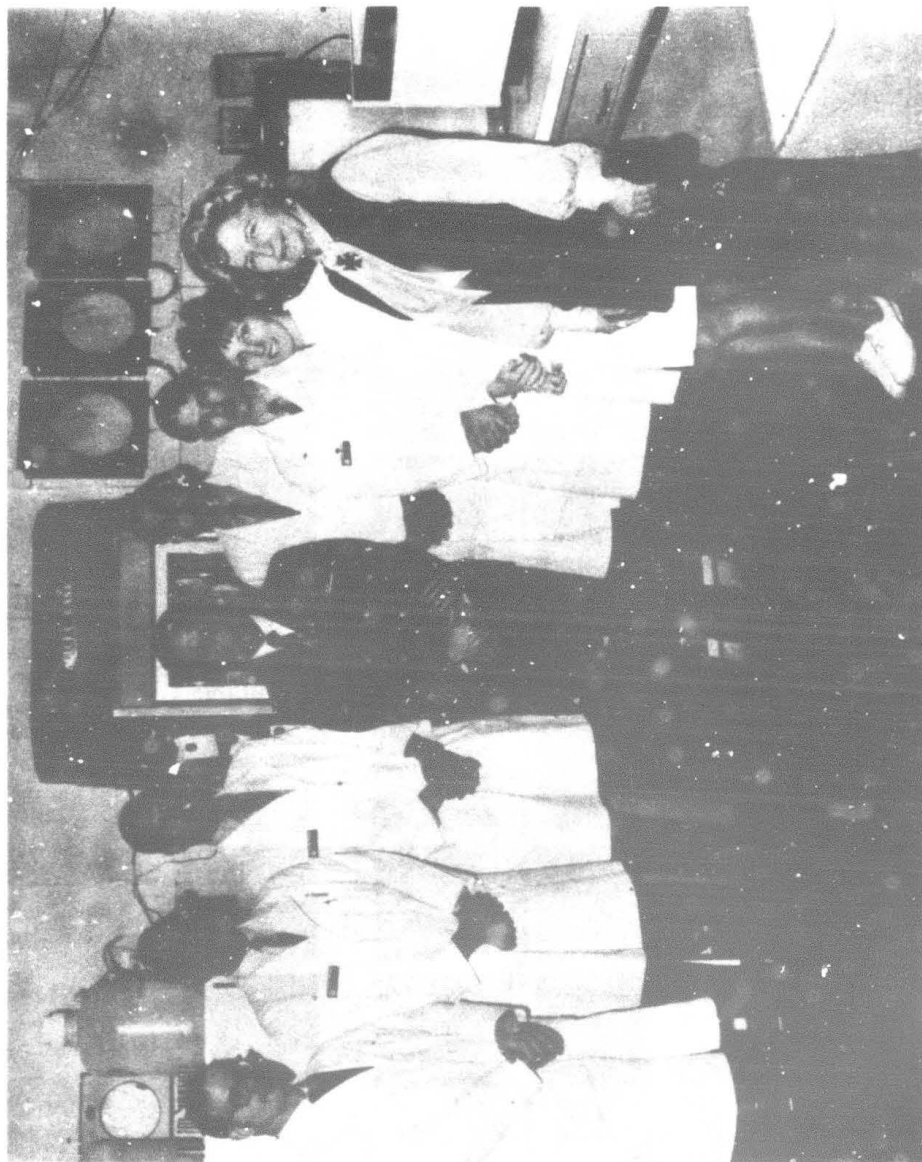


CONSULTANTS, ADVISORS AND FRIENDS

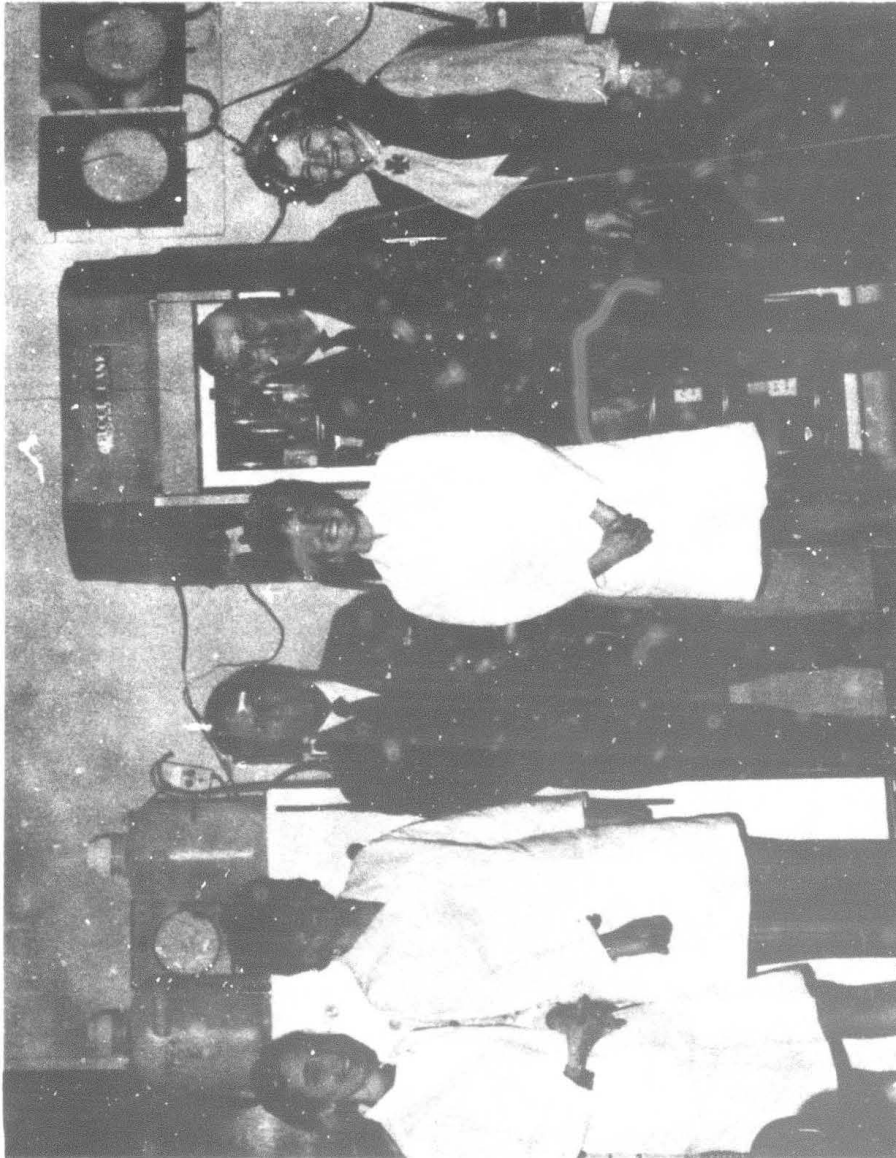


1958 - 1965





Left to right: J. A. Maples, A. G. Cumuze, Jr., R. G. DeBonville, R. F. C. MacPherson, L. R. McKinley, Jr., J. H. Young, Margaret E. McPeak, and Elise N. Hayes.



Left to right: Ima G. Shirley, Lillian W. Necessary, R. F. C. MacPherson, Margaret E. McPeak, F. R. Camp, Jr., and Elise N. Hayes.



Left to right: Margaret E. McPeak, Elise N. Hayes, and R. F. C. MacPherson.

A N N E X M

**HEPATITIS CARRIERS AMONG SOLDIERS WHO HAVE RETURNED FROM VIETNAM.
AUSTRALIA ANTIGEN STUDIES**

AD _____

REPORT NO. 939

HEPATITIS CARRIERS AMONG SOLDIERS WHO HAVE RETURNED FROM VIETNAM.
AUSTRALIA ANTIGEN STUDIES.

(Progress Report)

by

Baruch S. Blumberg, M.D.*
W. Thomas London, M.D.*
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***Commanding Officer/Director
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Fort Knox, Kentucky 40121

14 July 1971

Military Blood Banking: Automated Methodology
Work Unit No. 158
Combat Surgery
Task No. 00
Combat Surgery
DA Project No. 3A062110A821

Approved for public release; distribution unlimited.

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ABSTRACT

HEPATITIS CARRIERS AMONG SOLDIERS WHO HAVE RETURNED FROM VIETNAM.
AUSTRALIA ANTIGEN STUDIES.

OBJECTIVE

To determine the frequency of Australia antigen and antibody in military personnel returning from Vietnam.

METHODS

The first step was to test the hypothesis that service in Vietnam resulted in a significant increase in detectable Australia antigen and antibody. This could be done by testing soldiers before and after their service in Vietnam. The logistics of this plan are complicated, and it was decided to modify the study to test recruits prior to service and to test a different group of soldiers recently returned from Vietnam. We tested the null hypothesis that there was no significant difference in Au(1) and anti-Au(1), and both in these two groups. Blood was collected from 846 recruits at the US Army Reception Center in Fort Knox, Kentucky, who had not been to Vietnam and 803 members of the 194th Armored Brigade; the latter were collected within 1-18 months (average about 8 months) of their return from Vietnam. These blood specimens were used for the determination of red blood cell groups, the remaining sera were coded and sent to Philadelphia for Australia antigen and antibody testing. Australia antigen and antibody were detected by the immunodiffusion method. After the tests were completed the code was revealed and the distribution of antigen and antibody determined.

RESULTS

Australia antigen or antibody was detected by immunodiffusion in 17 of 803 Vietnam returnees, but in none of 846 recruits. From this it is estimated that about 58,000 hepatitis carriers will have been added to the population by servicemen returning from Vietnam. A past history of hepatitis appeared to be no more common among those with Australia antigen (Au(1)) or antibody (anti-Au(1)) than in those in whom they were not found. It is recommended that all servicemen returning from Southeast Asia be tested to detect asymptomatic hepatitis carriers.

HEPATITIS CARRIERS AMONG SOLDIERS WHO HAVE RETURNED FROM VIETNAM. AUSTRALIA ANTIGEN STUDIES.

INTRODUCTION

Australia antigen (Au(1)) is an infectious agent which can cause hepatitis in man (1,2). The detection of Australia antigen can be used to diagnose acute and chronic hepatitis and to detect hepatitis carriers. The latter observation is the basis of the present use of the Australia antigen test in many blood banks and laboratories to decrease the incidence of posttransfusion hepatitis.

In our initial study of Australia antigen we reported a high frequency of Au(1) in the indigenous populations of Vietnam (3,4), and a frequency of about 6% was reported in subsequent studies (5). This made it probable that US soldiers living under field conditions in Vietnam would have a much greater exposure to Australia antigen (and hepatitis) than similar troops stationed in the continental United States or in Europe, where the frequency of Au(1) in the general population is about 0.1%. There are many individuals who are (relatively) asymptomatic carriers of persistent Australia antigen. There are several studies which support the hypothesis that this persistent carrier state is genetically determined and controlled by a gene designated Au¹ (5,6,7). The frequency of the gene determining this trait varies from population to population and is very common in portions of the tropics and Southeast Asia, such as Vietnam. An implication of this hypothesis is that individuals with genotypes different than the one which determines the carrier state would be likely to contract acute hepatitis when exposed to the agent.

In 1968 we began to test soldiers who had returned to Philadelphia from Vietnam and were being treated at local military hospitals (for example, Valley Forge General Hospital). Hepatitis was common among these patients and Australia antigen was common in the patients with hepatitis. It was known that hepatitis was a major cause of morbidity in Vietnam (Table 1). These two findings made it likely that the hepatitis carrier rate and hence the frequency of Australia antigen among apparently asymptomatic Vietnam returnees would be greater than in US soldiers who had not been to Vietnam. We tested this hypothesis in a study of Armed Forces personnel located at Fort Knox, Kentucky.

MATERIALS AND METHODS

The first step was to test the hypothesis that service in Vietnam resulted in a significant increase in detectable Australia antigen and antibody. This could be done by testing soldiers before and after their service in Vietnam. The logistics of this plan are complicated, and it was decided to modify the study to test recruits prior to service and to test a different group of soldiers recently returned from Vietnam. We tested the null hypothesis that there was no significant difference in Au(1), anti-Au(1), and both in these two groups.

Blood was collected from 846 recruits at the US Army Reception Center in Fort Knox, Kentucky, who had not been to Vietnam and 803 members of the 194th Armored Brigade; the latter were collected within 1-18 months (average about 8 months) of their return from Vietnam. These blood specimens were used for the determination of red blood cell groups, the remaining sera were coded and sent to Philadelphia for Australia antigen and antibody testing.

Australia antigen and antibody were detected by the immunodiffusion method (8). After the tests were completed the code was revealed and the distribution of antigen and antibody determined.

The probability values for Tables 2 and 3 were determined by Fisher's exact method.

The estimates of Table 4 were determined as follows. The "most likely" entries are the products of the expected number of Vietnam returnees (2.6 million) and the fraction of cases having the given characteristics. The "minimum estimates" were obtained as 90% lower one-sided confidence bounds based on the normal approximation to the binomial distribution. The lower bounds on the estimated proportions were multiplied by the expected number of returnees to obtain the tabulated values.

RESULTS

The results are shown in Table 2. There were six individuals with Au(1) and 11 with antibody; all were Vietnam returnees. The probability that this distribution could be due to chance is 0.132×10^{-1} for antigen, 0.352×10^{-3} for antibody, and 0.440×10^{-5} for both combined (Fisher's exact method). Hence, the null hypothesis was rejected for antigen, antibody, and both; that is, it is highly likely that service in Vietnam leads to a significant increase in Australia antigen and hepatitis carriers, and in individuals with anti-Au(1).

Of the 17 individuals who had either Au(1) or anti-Au(1), only one gave a history of hepatitis or jaundice (Table 3). (He had been hospitalized for hepatitis in Vietnam about 4 months prior to the collection of his serum.) Of the 786 soldiers *without* Au(1) or anti-Au(1), 28 gave a history of hepatitis or jaundice; these groups are not significantly different from each other. From this, we can conclude that the persistent antigen state, or detectable antibody, is not, in this study, associated with clinical hepatitis.

In order to determine if a known hepatitis exposure could account for the presence of antigen or antibody a second questionnaire was sent to the 17 individuals with Au(1) or anti-Au(1). They were asked (again) if they had had hepatitis, or if they had transfusions. Replies were received from 12, including all six of the individuals with Au(1), and six of those with anti-Au(1). Of these, only one (a person with Au(1)) gave a history of hepatitis, and none had had transfusions. Similar inquiries

were sent to 17 soldiers randomly selected from the 194th Armored Brigade who had returned from Vietnam and 17 recruits from the US Army Reception Center who had not been to Vietnam. None gave a history of blood transfusion or hepatitis. From this, we could conclude that the presence of Au(1) and anti-Au(1) was not associated with a history of hepatitis or of transfusion.

DISCUSSION

From these results it is possible to conclude that service in Vietnam significantly increases the frequency of Australia antigen and of hepatitis carriers. It is possible to estimate the total number of individuals among those returning from Vietnam who will develop persistent Australia antigen and antibody and become carriers. In addition, confidence limits can be established for each of these estimates; the results are shown in Table 4. These estimates are based on the assumption that the exposure rate will be about the same during the entire period in which the Armed Forces have (and will) serve in Vietnam and that about 2.6×10^6 individuals will return from there. Because of the small sample size, the variance is large; this can be decreased by the study of larger numbers of individuals. This is now in progress.

The estimate of the number of hepatitis carriers is based on the assumption that the detection of Australia antigen by the immunodiffusion method identifies about one-third of possible carriers. There are, however, few data to support this estimate. It can be recalculated when better data are available.

From these calculations it can be said that the Vietnam experience will have added about 58,200 carriers to the US population. Each of these individuals can potentially spread hepatitis to people to whom they donate blood or with whom they come into contact.

The results can also be interpreted to mean that the individuals who became carriers usually do not have a history of hepatitis. According to concepts we have presented elsewhere, many of the carriers are individuals homozygous for the gene Au^1 which, according to this view, controls the development of the chronic antigenemic state. These individuals are (generally) asymptomatic while those with alternate phenotypes would be more likely to develop acute hepatitis when exposed. The latter are represented by the returnees listed in Table 3 who have a history of hepatitis but did not develop persistent antigen.

There are several recommendations that could follow from these observations.

1. Servicemen returning from Southeast Asia should be tested for the presence of Australia antigen and antibody and should be informed of the results. They can then refrain from donating blood, or from undertaking occupations, such as food handling, which could result in the spread of hepatitis.

2. One out of the 11 antisera was sufficiently potent to be used for routine Australia antigen testing. If we estimate that about 10% of antibodies detected by immunodiffusion are useful as reagents, then about 3,500 individuals whose sera could be used for testing could be identified. If only a fraction of these would donate or sell their blood sufficient antisera would be available for national and international laboratory use as Australia antigen test reagents. This is based on the assumption that the antisera detected in this program have as wide a specificity as antisera from transfused patients.

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TABLE 1

The incidence rates of viral hepatitis in U.S. Armed Forces stationed in the Republic of Vietnam (RVN), Europe (USAREUR), continental United States (CONUS), and world wide for the census years (CY) 1965 to 1970.* Rates per 1000 average strength per year.

CY	World Wide	RVN	USAREUR	CONUS
1965	1.00	5.70	0.35	0.50
1966	1.28	3.99	0.47	0.62
1967	2.41	7.01	0.25	0.97
1968	3.37	8.59	0.23	1.83
1969	3.02	6.41	0.35	1.98
1970	4.04	7.24	0.39	3.34

* Data obtained from the Analysis, Reports, and Publication Division, OTSG, Washington, D. C. 20314.

TABLE 2

Frequency of Au(1), anti-Au(1), and either in recruits and soldiers returned from Vietnam. The p value is determined by the Fisher's Exact Test.

Study Group	Total	Au(1)		Anti-Au(1)		Either	
		N	%	N	%	N	%
Recruits	846	0	0	0	0	0	0
Returnees	803	6	0.75	11	1.37	17	2.12
p value		0.132×10^{-2}		0.352×10^{-3}		0.446×10^{-5}	

TABLE 3

Responses to the question "Have you had hepatitis or jaundice?" by 803 returnees from Vietnam. The data are arranged in a two by two contingency table to distinguish between soldiers who did and did not have Au(1) or anti-Au(1).

		Hepatitis or Jaundice	
Au(1) or Anti-Au(1)		Yes	No
	Yes	1	16
	No	28	758

$$p = .468$$

TABLE 4

The most likely estimate and the minimum estimate (90% confidence) of the number of individuals 1) with persistent Au(1), 2) with anti-Au(1), 3) with either, 4) who are hepatitis carriers, among the estimated 2.6×10^5 individuals who have or will have returned from Vietnam. The computational methods used are discussed in the text.

Characteristic	Most Likely Estimate	Minimum Estimate
1. Au(1)	19,400*	9,522
2. Anti-Au(1)	35,616	21,966
3. Au(1) or anti-Au(1)	55,016*	38,138
4. Hepatitis carriers**	58,200	40,896

* Includes an estimated 3100 individuals who would have Au(1) prior to their residence in Vietnam. This is based on the estimate of 1/1000 Au(1) carriers in the general population.

** Calculated on the assumption that the immunodiffusion test detects about one-third of the hepatitis carriers.

A N N E X N

RINGER'S SOLUTION WITH LACTATE (pH 8.5)

80

245<

RINGER'S SOLUTION WITH LACTATE (pH 8.5)

Dillon *et al* (1), found that Ringer's solution with lactate (pH 8.5), when given in proper quantity, is as effective as autologous blood, given in volume equal to the volume of the hemorrhage, in saving the lives of lightly anesthetized, healthy dogs from which 40-75% of the blood has been removed, regardless of the duration (up to 150 minutes) or the degree of hemorrhagic hypotension (down to 30 mm Hg for one hour) that accompanies the hemorrhage. They also found that giving back all of the blood removed to induce shock, plus an equal volume of Ringer's solution with lactate (pH 8.2), and giving as little as one-half of the blood removed to produce shock, plus Ringer's solution with lactate pH 8.5 per formulary volume, are equally effective methods of treating hemorrhagic shock of the Wiggers type and are superior to the ten other forms of treatment investigated. In their series of experiments, Dillon *et al*, in agreement with the experience of Shires *et al* (2), and Wolf (3), demonstrated the superiority of giving blood together with Ringer's solution with lactate over giving blood alone in treating hemorrhagic shock. In addition, this series of experiments shows that the transfusion of blood is an indispensable part of the treatment of hemorrhagic shock because the giving of only one-half of the blood removed to produce shock significantly increases the effectiveness of treatment with Ringer's solution with lactate. They conclude that "evidently, the negative load of hemoglobin effected by dilution of the red cells remaining after the hemorrhage needed to produce and sustain hypotension for 150 minutes, by the Ringer's solution with lactate, is biologically significant."

Hypertonic Glucose. McNamara *et al* (4) have suggested that hypertonic glucose may prove a valuable adjunct in the acute resuscitative therapy of hemorrhagic shock.

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A N N E X 0

**COMPARISON STUDIES OF WHOLE BLOOD STORED IN
ACD AND CPD AND WITH ADENINE**

AD _____

REPORT NO. 719

**COMPARISON STUDIES OF WHOLE BLOOD STORED IN
ACD AND CPD AND WITH ADENINE**

Progress Report

by

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and
Lt Colonel F. R. Camp, Jr., MSC**

**Blood Transfusion Research Division
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121**

21 March 1967

**Study of Bank Blood Preserved in Acid-Citrate-Dextrose
and Acid-Citrate-Dextrose with Adenine After
Forty-two Days of Storage
Work Unit No. 155
Combat Surgery
Task No. 00
Combat Surgery
DA Project No. 3A025601A821**

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ABSTRACT

COMPARISON STUDIES OF WHOLE BLOOD STORED IN ACD AND CPD AND WITH ADENINE

OBJECTIVE

Evaluation of the in vivo post-transfusion survival of ACD or CPD with or without adenine and stored for 25, 35 or 42 days.

METHODS

Single-unit autotransfusions were given to normal volunteers and the survival of the transfused erythrocytes was measured by dual isotope techniques.

RESULTS AND CONCLUSIONS

Using 70% post-transfusion survival as the criteria, the average survival of the four different types of solutions was over 70% at 28 days. The ACD and CPD solutions were below this at 35 and 42 days. However, the solutions with adenine had values over 70% for 35 and 42 days. There was no statistical difference between the two basic anticoagulant solutions with adenine, but the survival values were significantly higher than the non-adenine containing units in every instance. Other chemical determinations failed to show changes of a toxic nature, and no toxic effects were observed in the recipients.

Since all units containing adenine had survival values greater than 70% in the 28- and 35-day periods, these units would seem to indicate effective preservation and that whole blood preserved in adenine-supplemented anticoagulants could be used after 35 days storage, and after 42 days in an emergency.

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COMPARISON STUDIES OF WHOLE BLOOD STORED IN ACD AND CPD AND WITH ADENINE

INTRODUCTION

The goal of transfusion therapy is replacement of fluid volume and functioning erythrocytes with their oxygen carrying capability intact. To achieve this goal, whole blood is collected into a suitable anticoagulant media that will preserve the red blood cells, yet be relatively innocuous when transfused into the recipient. One measure of the preservative effects of a solution would be the number of stored red blood cells that remained in circulation after transfusion.

The anticoagulant solution, acid-citrate-dextrose (ACD), has been commonly used for whole blood storage since World War II because it is relatively non-toxic in the recipient, but it has a suboptimal preservative effect (11, 19, 3, 13). As measured by methods using radioisotope labeled red blood cells, blood stored in ACD in glass containers over 21 days had 70% or less of the cells still remaining 24 hours after the transfusion. This low post-transfusion survival would indicate less effectiveness as replacement therapy; therefore, storing ACD blood beyond 21 days has been considered undesirable (19). This 21-day storage limitation has stimulated numerous efforts to produce new anticoagulant solutions leading to more effective blood preservation. An alternate solution, citrate-phosphate-dextrose (CPD), was designed to provide an external environment that is better tolerated by the red blood cells and is considered to have a useful storage period of 28 days (8).

The internal energy of the red blood cell has also been studied to determine if this might be an area sensitive to storage effects. Adenosine-triphosphate (ATP), the basic energy source for cellular metabolism and linked with the maintenance of the cell wall, appeared vulnerable and various chemicals have been added to help maintain the ATP level during storage (5, 15, 2, 14, 12, 7, 18, 17, 20). Though higher ATP levels could be observed, adenine, in particular, was more effective in increasing post-transfusion survival in blood stored over 35-46 days (7, 17).

The present study used the in vivo post-transfusion survival percentage to determine the effect of adenine on the survival percentage over selected storage periods, and to evaluate this in ACD or CPD as the basic anticoagulant solution.

MATERIALS AND METHODS

Full-unit (450 ml) autotransfusions were used in all studies. The subjects were normal male volunteers, 18 to 43 years of age. Units were drawn into plastic containers using standard blood bank refrigerators for the various selected time intervals. Two weeks before transfusion, a sample of blood was obtained under sterile conditions and aliquots placed in thioglycollate media and cultured at 4, 25 and 37°C. On the day of transfusion, another blood sample from the unit was examined microscopically for bacteria.

In vitro analytic procedures were done on blood samples obtained from the subject before and after the transfusion and from the unit on the day of transfusion. Measurements were made of the glucose, lactate, uric acid, plasma hemoglobin, sodium, potassium, pH and bilirubin levels. Micro hematocrits and urinalysis were performed. Coagulation tests included clotting time, prothrombin time, partial thromboplastin time and platelets. Osmotic fragility was measured by the osmogram method (16).

In vivo testing involved dual isotope measurement of post-transfusion survival. Fresh cells from the recipient on the day of transfusion were labeled with phosphorus³² using methods modified from Mollison (13) and Berlin (1). The stored unit was labeled with chromium⁵¹ using 50 microcuries, incubated for 2 hours at 25°C and the reaction stopped with ascorbic acid. Appropriate counting systems were used measuring the pre-infusion standards and the post-transfusion samples which were obtained at 15 and 30 minutes, 24 hours and approximately three times per week for three weeks. The 100% activity was calculated from the immediate post samples and the follow-up samples expressed from this as a per cent (10, 4). The percentage points were graphed on semilog paper against time and the 24-hour post-transfusion survival percentage and $T_{1/2}$ determined from a line drawn from the points.

The study was set up in two phases. In both, all subjects in the 42-day storage period had two units removed 42 days apart. One was collected in the basic anticoagulant, the other had the same anticoagulant plus adenine (0.5 μ M, prepared sterilely by Fenwal Co.) in a randomized sequence. This was to determine if adenine would improve the survival value, using the same person as a control.

The first phase involved 33 transfusions and 19 subjects using blood stored for 42 days in either ACD or ACD and adenine. Thirty-

seven units were cultured; however, five had a question of contamination and these units were not given. Of the remaining units transfused, 26 units could be included as the matched pair studies.

In the second phase, 123 transfusions were given and included CPD and CPD with adenine (0.5 μ M, Fenwal Co.) and storage periods of 28, 35 or 42 days. As before, the 42-day group consisted of two units and 24 ACD and 28 CPD units could be paired. In this group of over 600 separate cultures, two were positive and these two units were not given. Repeated cultures of these units were negative, suggesting contamination during the sampling procedure.

RESULTS

The average post-transfusion survival percentages for the first phase of 42-day-old ACD blood was 49% and 70% when adenine was present. In the second phase a similar highly significant difference ($p < .001$) was found at all storage periods (see Fig. 1), though the

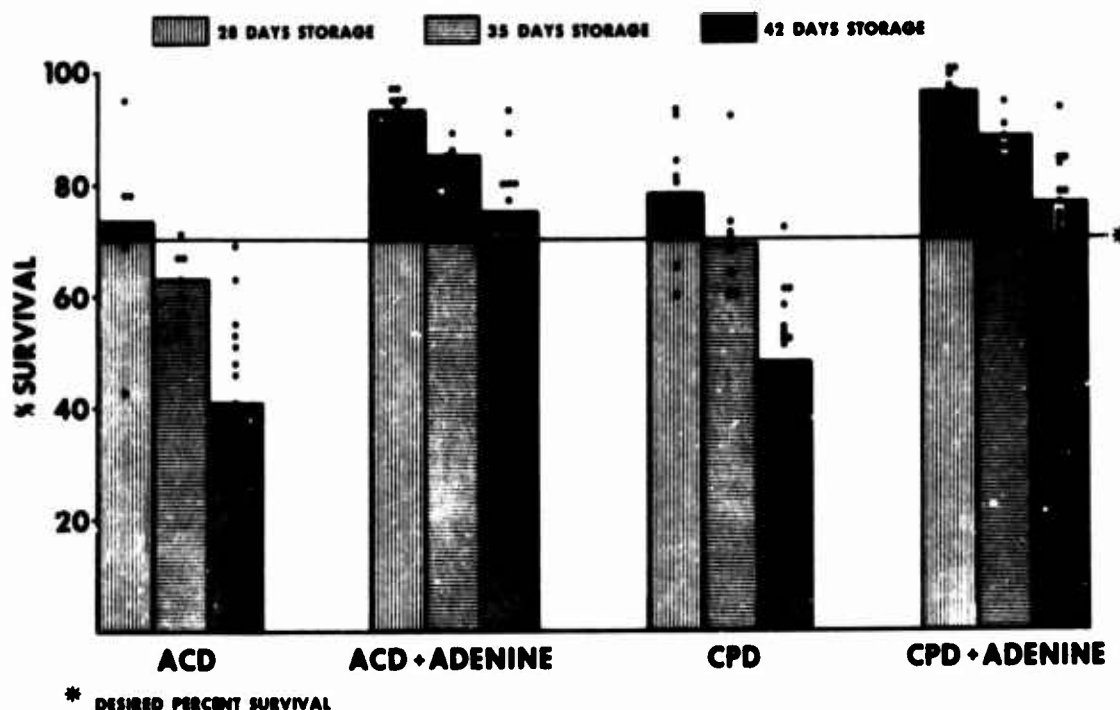


Fig. 1. Summary of survival percentages for each solution for each storage period. Average survival for adenine units was over 70% at all time periods. Individual values are represented and none of the adenine units were below 70% at 28 or 35 days. Comparing the basic anticoagulants, ACD and CPD, did not show significantly different survivals.

high survival levels present when adenine was added to ACD and CPD did not represent a statistically significant difference between the two anticoagulants. The non-adenine containing blood did have over 70% survival at 28 days, but fell below this at 35 or 42 days. Again, there was no statistical difference between the ACD and CPD anticoagulant solutions. In several instances, survival values for specific units without adenine were still able to approach the 70% level even at 42 days. It should be noted that all adenine-supplemented units had over 70% survival after 28 or 35 days, and only a few fell below this value even after 42 days.

In the paired studies, 12 of the 13 pairs from the first phase (Fig. 2), and all in the second phase (Fig. 3--next page) had higher

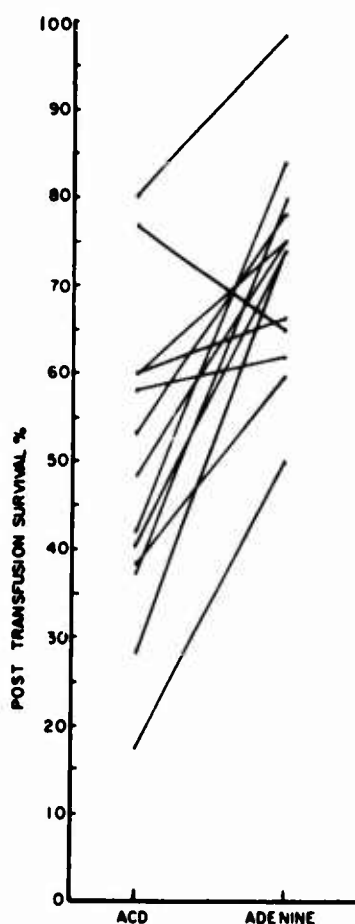


Fig. 2. Phase 1. Comparison of survival percentages of two units from same subject. Twelve of 13 showed higher survival when adenine was present.

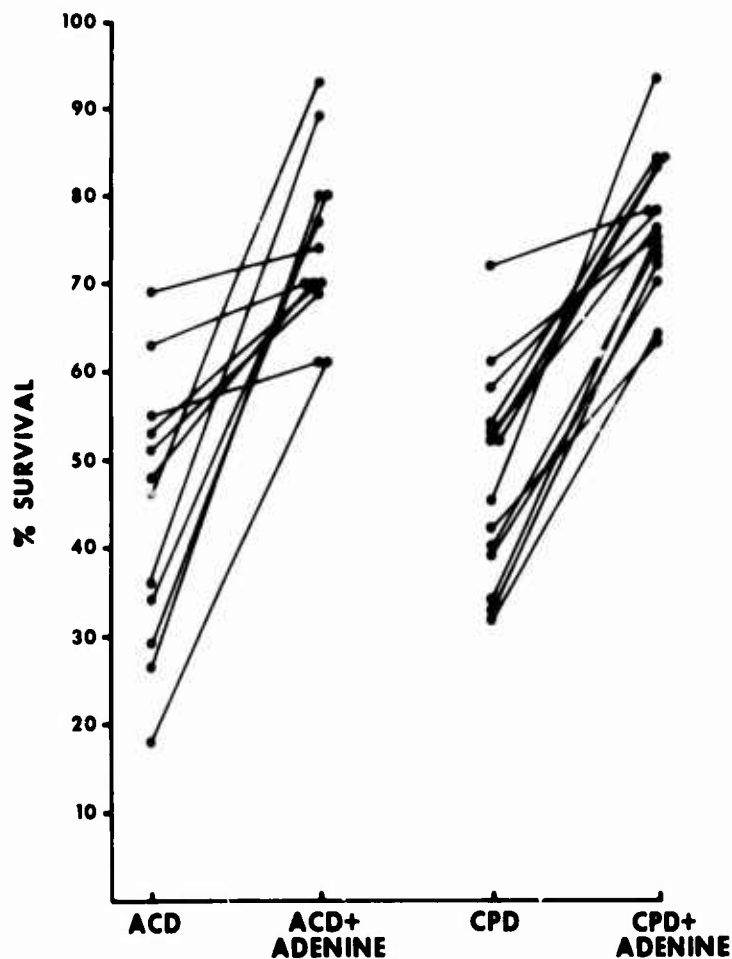


Fig. 3. Phase 2. Comparison of survival of two units from same subject using two anticoagulants. In all cases, higher survival was noted when adenine was present.

values when adenine was present. Not all units had the same increase in value, and though the basic trend toward improved survival values was highly significant ($p < .001$), some moderate variation of pattern did occur that was not statistically significant.

During the first phase, six subjects reported subjective symptoms ranging from stiffness to fever. Four received ACD, the others adenine solutions and no correlation could be made. These occurred and subsided in the first 24 hours, and did not occur when a second unit was given. With the second phase, the subjects remained under observation in the hospital until their release the day after the transfusion.

During this period, only one individual had symptoms which consisted of a transient rash during the night that disappeared by morning.

Despite extensive in vitro studies, little evidence could be obtained that adenine had caused any toxic accumulations; for example, there was no change in uric acid levels. The test results of the units followed the same patterns as previously described during storage, including elevations of lactate, plasma hemoglobin, potassium, and decreased levels of glucose, pH, and the clotting factors, as well as increased osmotic fragility which correlated with the survival percentages (see Table 1--next page). The alterations in the recipient were minimal with the only changes being the rising plasma hemoglobin and bilirubin which did correlate with the survival values. Comparisons of the average level of the plasma hemoglobin did show a significantly higher level ($p < .05$) for the CPD containing units compared to ACD units (Fig. 4, page 8).

DISCUSSION

In vitro and in vivo tests represent two different approaches in determining the usefulness of stored whole blood. In vitro studies of the stored unit have been used but the specific tests presently available have not shown significant discrimination in the evaluation of the storage effects in a transfusion (19, 3, 13). In contrast, the in vivo methods, measuring how much of the transfused blood is still in circulation, are closer to the physiological situation. One type of in vivo test, the isotope technique, has been useful in detecting the changes in survival after transfusion of small amounts of blood.

This particular method was applied in the present study using phosphorus³² labeled fresh cells as a corrective factor for the survival determination of the chromium⁵¹ tagged stored cells. It was observed that the most vulnerable cells are destroyed in the circulation immediately, with the major loss occurring in the first 24 hours (9). Those cells remaining in the circulation after this tend to have life spans similar to normal circulating cells suggesting that the damage during storage is either no longer severe enough to cause cell loss or it has been corrected. Using these methods, ACD blood stored in glass containers for 21 days will usually have only 70% of the cells surviving. This figure has been used as a guideline (3) and new solutions or additives must meet this level over longer storage periods before they can be considered as effective replacements.

TABLE 1
Summary of in vitro Changes with Storage

Analysis of units and recipients are listed under each basic anticoagulant solution.
Storage periods of 28, 35 or 42 days are listed horizontally for each test performed.

ITEM	BASELINE	Storage Period (Days)	ACD			ADP			CPO		
			No. Adm.	Stored Unit	Subject After	No. Adm.	Stored Unit	Subject After	No. Adm.	Stored Unit	Subject After
Plasma Hemoglobin 4.0 (2.7)		28	51(5)*	54(9)	2.6(1.4)	60(12)	62(2.9)	56(13)	3.0(1.1)	3.0(1.1)	3.0(1.1)
		35	82(17)	1.00(26)	2.8(1.0)	100(25)	4.0(1.0)	130(37)	3.7(1.3)	3.7(1.3)	3.7(1.3)
		42	143(29)	1.35(24)	6.9(1.2)	109(22)	12.2(2.5)	172(25)	8.0(2.7)	8.0(2.7)	8.0(2.7)
Bilirubin .6 (1.1)		28	.6(1.3)	.5(1.2)	.8(1.2)	.5(1.1)	.5(1.1)	.2(1.2)	.9(1.1)	.9(1.1)	.9(1.1)
		35	.3(1.1)	.4(1.2)	1.4(1.3)	.4(1.2)	.4(1.2)	2.5(1.3)	1.0(1.3)	1.0(1.3)	1.0(1.3)
		42	.4(1.1)	.3(1.1)	1.1(1.2)	.5(1.2)	.5(1.2)	2.2(1.4)	1.5(1.5)	1.5(1.5)	1.5(1.5)
Glucose 105(9)		28	247(20)	282(30)	83(7)	171(11)	83(15)	170(9)	103(12)	103(12)	103(12)
		35	225(12)	239(16)	80(3)	159(10)	81(14)	164(7)	85(6)	85(6)	85(6)
		42	247(40)	254(22)	105(10)	158(9)	92(9)	160(12)	92(9)	92(9)	92(9)
Uric Acid 5.0(4)		28	2.3(1.2)	2.8(1.2)	5.0(1.3)	2.8(1.3)	2.8(1.3)	3.4(1.2)	5.0(1.3)	5.0(1.3)	5.0(1.3)
		35	3.4(1.2)	4.8(1.3)	4.3(1.3)	3.0(1.2)	4.7(1.3)	3.1(1.2)	4.3(1.3)	4.3(1.3)	4.3(1.3)
		42	3.0(1.4)	3.3(1.4)	5.5(1.5)	3.2(1.2)	5.8(1.5)	3.3(1.3)	5.9(1.4)	5.9(1.4)	5.9(1.4)
Potassium 4.2(2)		28	17(1)	18(1.7)	3.7(1.1)	20(1.5)	3.9(1.2)	17(1.1)	3.8(1.1)	3.8(1.1)	3.8(1.1)
		35	25(2)	21(1.8)	3.8(1.1)	23(1.2)	3.9(1.1)	23(1.1)	3.6(1.1)	3.6(1.1)	3.6(1.1)
		42	28(1.3)	23(1.6)	3.9(1.4)	24(1.1)	4.0(1.1)	24(1.8)	3.8(1.1)	3.8(1.1)	3.8(1.1)
pH 7.30(1C)		28	6.57(1.1)	6.59(1.1)	7.39(1.1)	6.53(1.1)	7.38(1.1)	6.54(1.1)	7.39(1.1)	7.39(1.1)	7.39(1.1)
		35	6.49(1.1)	6.49(1.1)	7.41(1.1)	6.45(1.1)	7.38(1.1)	6.52(1.1)	7.38(1.1)	7.38(1.1)	7.38(1.1)
		42	6.50(1.1)	6.54(1.1)	7.33(1.1)	6.44(1.5)	7.34(1.1)	6.45(1.1)	7.36(1.1)	7.36(1.1)	7.36(1.1)
Osmotic Fragility % Point 17.7(1.1)		28	11.3(1.4)	12.6(1.4)	16.4(1.8)	10.5(1.9)	15.1(1.7)	11.0(1.6)	16.2(1.8)	16.2(1.8)	16.2(1.8)
		35	9.5(1.1)	11.9(1.4)	18.2(1.5)	9.6(1.7)	17.7(1.6)	10.2(1.0)	17.9(1.6)	17.9(1.6)	17.9(1.6)
		42	7.3(1.5)	9.9(1.8)	15.2(1.8)	7.2(1.4)	16.3(1.8)	9.3(1.1)	16.4(1.6)	16.4(1.6)	16.4(1.6)
Osmotic Fragility Slope .91(1.0)		28	.64(1.1)	.60(1.1)	.79(1.1)	.47(1.1)	.70(1.1)	.47(1.1)	.77(1.1)	.77(1.1)	.77(1.1)
		35	.43(1.04)	.56(1.06)	.81(1.07)	.41(1.06)	.92(1.06)	.47(1.05)	.92(1.06)	.92(1.06)	.92(1.06)
		42	.44(1.04)	.50(1.07)	.89(1.1)	.40(1.1)	.84(1.1)	.46(1.1)	.90(1.1)	.90(1.1)	.90(1.1)
Survival %		28	73(5)	73(5)	93(4)	78(5)	78(5)	94(7)	94(7)	94(7)	94(7)
		35	63(6)	63(6)	85(4)	69(6)	69(6)	88(6)	88(6)	88(6)	88(6)
		42	41(10)	41(10)	75(5)	48(8)	48(8)	76(5)	76(5)	76(5)	76(5)

* Standard deviation of the means

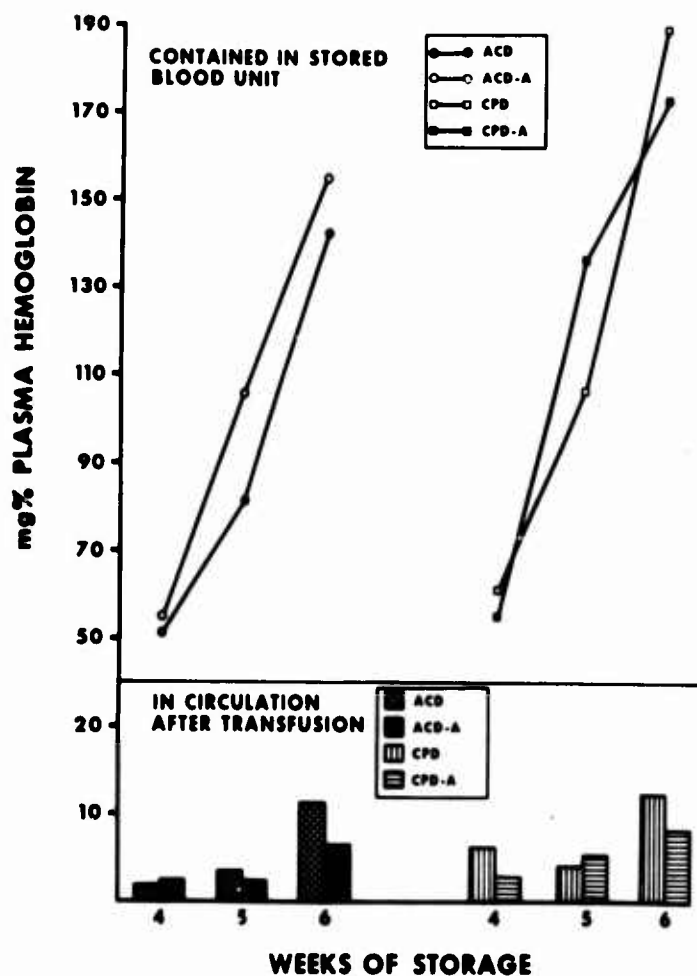


Fig. 4. The average plasma hemoglobin values are shown in relation to storage periods in the units and in the recipient. Note that the level in the recipient is not above the normal range until the unit average exceeds 120-130 mg %.

As part of the investigation of the site of damage occurring during storage, the internal metabolism of the red blood cell was considered. Adenosine-triphosphate (ATP) can be a primary energy source for the cell and thereby aid in maintaining the cell wall (3, 5, 15). Materials can be added which help maintain the ATP level and indirectly prolong cell life. Adenosine was one of the earliest to be tried, but some of the subjects developed hypotension (5, 12). Inosine was also tried, with an increase in storage time to 28 days (5, 12), while addition of adenine had 70% survival after 35-46 days of storage (7, 20).

The present studies tested adenine-supplemented anticoagulant solutions after various storage periods using both in vitro and in vivo tests. In vitro testing did not reveal any toxic accumulation related to adenine in these normal subjects. The uric acid concentration, a possible breakdown product of adenine, remained normal, though this may be a problem in the patient with high body pools or poor renal excretion of uric acid. Despite visible hemolysis in some units with occasional values of over 500 mg/100 ml plasma hemoglobin in the unit, the blood could be transfused without untoward reaction and with only minimal increase of the recipient's plasma hemoglobin level.

In vivo testing involved measuring survival of the transfused cells by isotope labeling methods. These results would appear to be meaningful since autotransfusions were used which reduced the chance of secondary effects from differences between bloods of two different persons, and the same subject could act as his own control in the two-unit phase. Though ACD solutions have been considered unable to maintain 70% survival much beyond 21 days, there were occasional individual units in ACD or CPD alone that approached the 70% level even after 42 days of storage. This broad range of observed survival percentages certainly suggested that there is an individual variation of resistance of various erythrocyte populations to storage damage as also observed in studies by Dern (6). There also appeared to be a different interaction of adenine with various populations since not all units had the same increase in survival after adenine. Often those with the lowest survival initially had the best increase, suggesting that adenine may have been more effective in these but it was secondary to some other factor in those units with the higher survivals even without adenine.

An interesting point was the over-all average being over 70% for ACD and CPD anticoagulants at 28 days with no statistical difference between them either by survival or chemically. Such survival at 28 days for CPD solutions had been reported (9) while this survival for ACD is better than expected. This may be a result of several factors. Generally, the original studies were made on blood collected in glass containers under vacuum while, presently, gravity filling of plastic containers is used. Such a difference between glass and plastic containers was noted by Simon (18) when he was testing ACD blood with adenine. As another factor in the present study, donors and recipients were healthy males receiving their own blood that had been collected and stored undisturbed under optimum conditions. However, these same comments also apply to the CPD units indicating that there seems to be little difference between anticoagulants which was observed even if adenine was present.

It is apparent that since adenine did significantly increase the survival value in both types of anticoagulants, it, per se, is a useful additive probably acting in metabolic systems involving ATP. Since 70% survival could be maintained in all units after 35 days, these units could be used in routine transfusion therapy, and the units stored for 42 days probably could be used in an emergency. However, it should be realized that the present work involved single-unit transfusions in healthy subjects, and additional work does remain to be sure that multiple units transfused into non-healthy individuals are without serious toxicity, before universal application of these findings is made.

SUMMARY

Using 70% post-transfusion survival as the criteria, ACD and CPD anticoagulant solutions with and without adenine were tested after storage for 28, 35 or 42 days. At 28 days, all solutions had average survivals of 70% or better. The ACD and CPD solutions were below this at 35 and 42 days. However, the solutions with adenine had values over 70% for 35 and 42 days. There was no statistical difference between the basic anticoagulant solutions with or without adenine. In comparing two units obtained from the same subject, survival percentages were significantly higher in almost every unit when adenine was present. Other chemical determinations did not show harmful changes and no toxic effects were observed in the recipients.

Since all units containing adenine had survival values greater than 70% in the 28- and 35-day periods, these units would appear to have been effectively preserved and blood stored under these conditions could be used in routine transfusions, reserving units stored 42 days for emergency use.

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A N N E X P

**STANDARDIZATION OF BLOOD TRANSFUSION REACTION STUDIES IN THE MILITARY.
DELEGATION OF RESPONSIBILITY FOR A MEDICAL TEAM CONCEPT.
ROLE OF THE HOSPITAL TRANSFUSION BOARD**

AD _____

REPORT NO. 867

STANDARDIZATION OF BLOOD TRANSFUSION REACTION STUDIES IN THE MILITARY.
Delegation of Responsibility for a Medical Team Concept.
Role of the Hospital Transfusion Board.

(Progress Report)

by

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ABSTRACT

STANDARDIZATION OF BLOOD TRANSFUSION REACTION STUDIES IN THE MILITARY.
Delegation of Responsibility for a Medical Team Concept.
Role of the Hospital Transfusion Board.

OBJECTIVE

To determine standards for managing blood transfusion reactions.

METHODS

The Hospital Transfusion Board was reviewed in its role of implementing and monitoring a system of transfusion reaction detection and management.

RESULTS AND CONCLUSIONS

One of the main goals of the Hospital Transfusion Board is the prevention of injury from blood transfusion therapy. To fulfill this mission, a delegated, coordinated procedure system for the investigation and management of Blood Transfusion Reactions (BTR) has been evolved. This includes the logical implementation of the cross-check principle in the patient at the operational level of the hospital ward and at the laboratory bench. The pathologist is provided with a hospital-wide detection and salvage system which will: (1) detect at the earliest possible moment the existence of a BTR (including Incompatible Hemolytic Blood Transfusion Disease (IHBTB)); (2) detect a probably coexistent consumption coagulopathy; (3) mobilize swiftly lifesaving therapy in diagnosed cases of IHBTB; and (4) develop a record of the events as data are recorded on the Blood Transfusion Reaction Report.

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STANDARDIZATION OF BLOOD TRANSFUSION REACTION STUDIES IN THE MILITARY.
Delegation of Responsibility for a Medical Team Concept.
Role of the Hospital Transfusion Board.

INTRODUCTION

Dr. William H. Crosby's editorial on the need for Hospital Transfusion Boards has provided the various hospital services a means of coordinating blood transfusion practices within their medical facilities (5). One of the main goals of the Hospital Transfusion Board continues to be that of preventing injury from blood transfusion therapy.

Subsequent to 1960, emphasis was placed on developing a system, designed to delegate responsibility, mainly for the creation of a medical team concept. The role of the Hospital Transfusion Board in implementing and monitoring a system of transfusion reaction detection and management is an imperative requirement; however, it can fulfill its mission only if supported with pertinent administrative specifications. Therefore, it is the purpose of this report to show the need of such a concept and to suggest a *total system* that is feasible for implementation in all treatment centers--military and civilian.

GENERAL CONSIDERATIONS (2)

In spite of meticulous care and close attention to procedural detail, it appears inevitable that a small number of undesirable reactions to transfusion may occur. Among the several classes of transfusion reactions, those which produce intravascular hemolysis are the most dangerous; this type may cause the death of the recipient. We have, for this reason, long accepted the responsibility for reexamination of tests on all patients in whom hemolytic transfusion reactions may be suspected. In our zeal to close all possible avenues of error, we have randomly evolved a time-consuming set of repetitive maneuvers and serologic tests that fail to lead quickly to answers that are necessary to care properly for these patients. There can be but little quarrel that the data required by Standards (4) are useful. It would appear, however, that the *first order of business* is to provide a "yes" or "no" answer as quickly as possible to the simple question, "Has a hemolytic transfusion reaction occurred?" An affirmative answer to the question means that the patient requires emergent treatment. Subsequently, the rechecks recommended by the Standards should be completed as promptly as possible, and may then be carried out without further compromising the recipient's chances of surviving a hemolytic reaction to transfusion.

The existence of Incompatible Hemolytic Blood Transfusion Disease (IHBTD) can be established quickly and beyond doubt by demonstrating first, a positive direct antiglobulin (Coombs) test, which appears during or *after* a transfusion, and secondly, the presence of elevated free hemoglobin in the serum or plasma of the recipient. In a recent reappraisal of the diagnosis and treatment of IHBTD, Nalbandian *et al* (9)

emphasize the need for a fresh approach to the problems of detection and salvage of victims of incompatible blood transfusions by clinicians and blood bank workers alike. In the interest of saving the lives of recipients receiving incompatible blood, these authors insist on the need for speed, simplicity, and precision by a technic of *cross-checking*. They contend, and we agree, that the chances of survival vary inversely with the *time* interval between the infusion of incompatible blood and the start of treatment. Treatment with mannitol, closely in accord with the recommendations of Barry and Crosby (1) and of Parry (13), has been endorsed. Nalbandian and associates emphasize the need for *speed* and propose that the simple visual inspection of posttransfusion serum is adequate to confirm significant hemoglobinemia. A direct antiglobulin test can be performed in a very few minutes on the same specimen of blood. We have thus fulfilled their criteria of speed and simplicity. Concerning precision: the diagnosis can be established or excluded with equal accuracy. Instructions for the diagnosis and treatment of IHBT and stocks of mannitol have been placed by Nalbandian and his associates at each nursing station throughout the hospital where blood transfusions may be given.

In our estimate, the salient areas to the avoidance of pitfalls concern:

1. Communication between personnel in the patient care area and the laboratory. This must be established from the moment a reaction is first suspected and maintained throughout the interval of observation and/or treatment.
2. Communication within the laboratory between bench workers and physician supervisor, irrespective of the hour or the day of the week a reaction is recognized.
3. Care with technic of obtaining samples required for the study.
4. Documentation of samples, including relationships in time to infusion of the offending transfusion and to each other.

As we have indicated above, once the diagnosis is established, treatment with mannitol must be prompt and directed toward maintaining urine output at approximately 100 ml/hr. With respect to technic, the single most significant pitfall to be avoided is the artificial induction of hemolysis in the first posttransfusion specimen of blood. This can be prevented only through careful venipuncture following minimal stasis and probing, and by the use of chemically clean and dry equipment.

The clear designation of time and date, along with proper patient and source of specimen identification, is the only means by which accurate reconstruction of events can be achieved. Properly organized, the blood bank laboratory will have saved all crossmatch specimens and pilot tubes for a minimum of 7 days following transfusion. These specimens,

along with samples from the container involved in the reaction and post-transfusion samples, now can be rechecked for group, Rh type, and compatibility testing. When the evidence suggests that a hemolytic reaction has in fact occurred, antibody titrations before and after transfusion, especially the pertinent natural, regular isoantibodies, will provide confirmatory data. Culture of pertinent blood specimens is also to be performed. Quantitative haptoglobin estimates under certain conditions provide another parameter of evaluation of a delayed, confirmatory nature.

When other accidents appear to be responsible for a hemolytic transfusion reaction, e.g., gross bacterial contamination or massive hemolysis of a donor unit, whatever the cause, the sequences of communication and notification of top level professional personnel are even more important because these patients may die in a very short time. For the latter problems, the nature of specimens and the way in which they are to be handled may, of course, be modified to fit the circumstances. The necessity for accurate documentation and labeling of materials to be studied remains the same. The method of Dr. Paul J. Schmidt, shown in Table I (16), summarizes the findings and treatment of transfusion reactions.

Since the occurrence of a transfusion reaction may represent a technical failure of the blood bank service, each such instance must serve as a teaching and reorientation exercise for all personnel in the department. It is only by this means that every member of the staff can rededicate himself to minimizing the risks of blood transfusion. This highlights the absolute necessity for maintaining continuous and effective training programs and/or workshops for all personnel engaged in blood grouping, blood banking, and blood transfusion practices.

SPECIFIC CONSIDERATIONS (10,11)

A Blood Transfusion Reaction (BTR), excluding late sequelae, may be defined as any adverse clinical sign or symptom occurring singly or in combinations, during or shortly after a blood or blood component transfusion, and the result of that transfusion. A summary of BTR's of several types is included in the Supplement. Potentially catastrophic is the IHBTD. This is defined as a condition incident to a blood transfusion in which intravascular hemolysis of sensitized erythrocytes is so accelerated as to portend a possible fatality.

PRINCIPLES OF DIAGNOSIS

In analyzing data, it is infinitely preferable to confirm results by methods other than those used initially. Thus, in investigating a possible IHBTD, one must use methods other than those by which compatibility of the donor and recipient blood was originally determined. Under these circumstances rapid *cross-checking of the parameters of pathophysiology in the patient* is needed, rather than the traditional, time-consuming recheck of the entire typing and crossmatching procedure that

may at best perpetuate original technical errors. In cross-check of a BTR, attention is directed only to those parameters of pathophysiology (6) *which would be diagnostically altered if IHBTB were present*. The two diagnostic parameters which also function selectively as early indices of severity are elevated plasma hemoglobin levels and positive direct Coombs test when compared with pretransfusion specimens.

Several papers (7,8,14,15) have emphasized the aspect of consumption coagulopathy in association with IHBTB. It is essential to investigate for consumption coagulopathy once IHBTB has been diagnosed.

It is prudent to do a gram stain on a centrifuged aliquot of plasma from the transfused unit to detect the rare case in which the transfusion reaction may be due to gross bacterial contamination of the unit of blood.

The two determinations crucial to the diagnosis of IHBTB, however, are the demonstration of elevated plasma hemoglobin levels and positive direct Coombs tests when compared with pretransfusion specimens.

PRINCIPLES OF THERAPY

Since 1953, a growing body of both evidence and opinion has supported the use of mannitol treatment in IHBTB (1,3,12,16). On the other hand, universal adoption of this therapy for IHBTB is not evident.

There is a point in time beyond which mannitol becomes progressively less effective. Thus, the interval between the offending transfusion and the initiation of mannitol therapy should be as short as possible. We recommend the classification of mannitol as an emergency drug and urge that it be stocked at all hospital stations where blood transfusions are given. At each station explicit directions for the intravenous use of mannitol should be posted (Table II). It is important to emphasize compliance with these measures; any oversight will increase the interval between transfusion and treatment, to the patient's detriment. Time saved in our proposed cross-check detection procedure and early recourse to mannitol therapy benefits the jeopardized patient, since effective treatment can be given much earlier.

When IHBTB has been diagnosed, it follows that mannitol therapy must be instituted and diagnostic procedures for a consumption coagulopathy carried out. If a significant coagulopathy is present, appropriate therapy is also added to the regimen. Probably in every case, and in variable degree, IHBTB is accompanied by a consumption coagulopathy. Because IHBTB may be superimposed on any number of very different underlying diseases, appropriate coagulation studies should be done to determine whether the coagulopathy is: (1) primary activation of the coagulation system with a secondary fibrinolytic component; (2) primary activation of the fibrinolytic system; or (3) coequal activation of both systems. Since treatment is quite different for each type of coagulopathy and fraught with disaster if treatment regimens are interchanged, the coagulopathy

must be categorized. Continuous, intravenous heparin is the indicated treatment for category (1); epsilon amino caproic acid for category (2); and heparin with or without epsilon amino caproic acid is acceptable for category (3), since Trasylol is no longer available by FDA edict.

The clinician should consult with the pathologist to obtain a profile of diagnostic and base line coagulation studies. *In consumption coagulopathy the basic pathophysiology is the intravascular conversion of plasma to serum. The central therapeutic principle is the titration of the patient by intravenous, indicated drug to revert the serum back to plasma as guided by the response of individual parameters in a periodically repeated coagulation profile.*

Because at present there are many different coagulation tests from among which pathologists may exercise personal preferences for the diagnosis of consumption coagulopathy, the profile of tests of coagulation parameters for diagnosis and for monitoring therapy is best left to the individual pathologist.

IMPLEMENTATION OF THE CROSS-CHECK PRINCIPLE

We now turn to the implementation of the principles of diagnosis and management of BTR and particularly IHBTB as discussed above. By segregating and separately specifying the duties and responsibilities of each member of the blood transfusion reaction investigative team (nurse, clinician, medical technologist, and pathologist), it has been possible to coordinate the *delegated activities* of several individuals located at various points in the hospital in order to detect early and salvage effectively victims of IHBTB. The mode of handling and recording the generated clinical and laboratory data on the Blood Transfusion Reaction Report form (Fig. 1) provides a document which to a great extent protects the physician and the hospital from the hazards of litigation.

There are four separate sets of instructions (see Supplement), one for each member of the professional team: (1) nurse; (2) physician; (3) anesthesiologist; (4) medical technologists. These instructions are posted at logical and appropriate stations throughout the hospital in segregated fashion such that the nursing staff only receive the nursing staff instructions; the physicians only receive the physicians' instructions, etc. (For the purpose of this report only, role-specific instructional procedures for professional personnel are attached hereto as a Supplement.)

We believe that the proper role of the pathologist is one of consultation in the circumstance of a BTR. A patient with the manifestations of one or more of the several types of BTR has acquired an additional potentially lethal complication superimposed on the underlying medical problem which must be diagnosed and treated in that clinical context by a clinician. The clinician must be supported by pertinent laboratory data and by consultations with the pathologist.

A diligent perusal of the figures and sets of instructions reproduced in the Supplement clearly demonstrates that this procedure system achieves multiple ends with maximum efficiency. While each member of the investigating team receives only his role-specific instructions, the composite result of the four different sets of instructions coordinates the several independent efforts to the extent that:

1. A BTR emergency (always a possible IHBTB) is recognized by the nurse who stops the transfusion and summons a physician. She then follows the seven instructions listed on the Blood Transfusion Reaction Report (Fig. 1).

2. The type of BTR is diagnosed and treated by the clinician with the benefit of instructions predicated on the cross-check principle. Life-threatening emergencies can be dealt with effectively because specific instructions and drugs are at hand.

3. There is created an historical record, the Blood Transfusion Reaction Report, which characterizes and summarizes the particular episode, and provides the hospital and involved physicians with documentary evidence in the event litigation occurs.

4. Rechecking (retyping and recrossmatching) is done *after* the life-threatening possibility of IHBTB has been appropriately treated.

SUMMARY

In order to have maximum detection and salvage of victims of incompatible blood transfusion the approach must be one of *cross-checking for evidence of incompatible hemolytic blood transfusion in the patient* rather than the traditional rechecking of *in vitro* specimens. Such an approach to this serious clinical problem yields more pertinent information in much less time.

Each episode of a BTR should be approached as a potential case of IHBTB. Since it has been shown that mannitol infusion is a lifesaving measure in the treatment of IHBTB when given soon enough, a rapid, accurate diagnosis of IHBTB is imperative. Application of the *principle of cross-checking in the patient* for the presence of this clinical problem directs attention to only two easily determined parameters of pathophysiology which would be diagnostically altered if IHBTB were present. These two parameters are: (1) plasma hemoglobin levels and (2) positive direct Coombs, when both are compared with pretransfusion specimens. If IHBTB is diagnosed, mannitol therapy is instituted immediately. Probable coexistent consumption coagulopathy must be diagnosed and treated in an appropriate manner.

We have evolved a delegated, coordinated procedure system for the investigation and management of the BTR's which is the logical implementation of the cross-check principle in the patient at the operational level

of the hospital ward and at the laboratory bench (see Supplement). The pathologist is provided with a *hospital-wide detection and salvage system*, which, if adopted *in toto* exactly as displayed in the Supplement, will: (1) detect at the earliest possible moment the existence of a BTR (including IHBTB); (2) detect a probably coexistent consumption coagulopathy; (3) mobilize swiftly lifesaving therapy in diagnosed cases of IHBTB; and (4) develop a record of the event as data are recorded on the Blood Transfusion Reaction Report. This summary record becomes part of the patient's hospital chart and to a great extent protects the hospital and physician from possible litigation. Most important of all, the death of patients from IHBTB hopefully may be eliminated.

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S U P P L E M E N T

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The Supplement contains a replication of four sets of instructions for investigation and management of BTR essential to the effective application of principles discussed in the body of this report.

ATTENTION: Nursing Staff

POST AT:

1. All Nursing Stations
2. Laboratory (Blood Bank, Chemistry, Bacteriology)
3. Postanesthesia Recovery Room
4. Operating Room
5. Emergency Room

ROLE OF NURSE IN MANAGEMENT OF BLOOD TRANSFUSION REACTION

PROCEDURE:

1. Members of the nursing staff have several immediate responses in connection with the detection of a blood transfusion reaction.
 - a. Stop the transfusion of blood, but leave the needle in the vein with a slow saline drip attached.
 - b. Notify immediately any available house physician, intern, attending physician, or anesthesiologist. The situation should be regarded as emergent and any available physician should be summoned.
 - c. Check for agreement of patient's name and number with the transfusion container labels and other records.
 - d. The nurse must follow seven explicit directions on the Blood Transfusion Reaction Report.
2. The nursing staff should assist the physician by carrying out any orders on a "stat" basis.
3. The nursing staff should determine that adequate supplies of mannitol are instantly available at the nursing station in case the physician should require its immediate use.

ATTENTION: Attending Physician

POST AT:

1. All Nursing Stations
2. Laboratory (Blood Bank,
Chemistry, Bacteriology)
3. Postanesthesia Recovery Room
4. Operating Room
5. Emergency Room

ROLE OF PHYSICIAN IN MANAGEMENT OF BLOOD TRANSFUSION REACTION

Pages 12-14: (1) Instructions.

Pages 15-17: (2) Differential Diagnosis of BTR.

Page 17: (3) Pertinent References.

A Blood Transfusion Reaction (BTR) is any adverse sign or symptom caused by blood or blood component transfusion and not related to the underlying disease of the patient manifested during or shortly after that transfusion.

An Incompatible Hemolytic Blood Transfusion Disease (IHBTB) is defined as that condition in which intravascular hemolysis of sensitized erythrocytes is so accelerated as to portend a possible fatality.

Early infusion of mannitol will save the lives of many patients with IHBTB. To test quickly for intravascular hemolysis, plasma is checked for pink or red color, a direct Coombs test is performed, and the plasma hemoglobin level is determined. Findings are compared with pretransfusion specimens, and if hemolysis is evident, mannitol is infused immediately (Table II). The shorter the interval between the offending transfusion and the use of mannitol, the more effective the treatment. To expedite therapy, all hospital stations where blood transfusions are given stock mannitol as an emergency drug with explicit directions for its use. Also, consumption coagulopathy must be anticipated and managed if IHBTB is present.

-
1. BTR, including IHBTB, is a disease and must be diagnosed and treated by a clinician with consultative support from the pathologist.

2. Any physician must respond when summoned by a nursing staff member to attend a potentially jeopardized patient who is having a possible BTR including IHBTB.
3. The physician shall determine whether a BTR of any type has occurred (see pages 15-17 for differential diagnosis of BTR). He must then institute appropriate therapy.
 - a. Among the several types of BTR, one which is potentially lethal is IHBTB and, therefore, must be recognized forthwith. In IHBTB, the diagnostic feature of intravascular hemolysis can be easily recognized. In IHBTB there will be:

- (1) Elevated levels of plasma hemoglobin.

- (2) Increased population of sensitized erythrocytes.

The former condition can be recognized by the determination of the plasma hemoglobin level (plasma turns pink at 20-30 mg/100 ml and above that becomes deeply red; normal plasma hemoglobin levels are 2-4 mg/100 ml); the latter condition can be recognized by the presence of a positive direct Coombs test which is quantitatively greater than the pretransfusion reaction specimen.

- b. Accordingly, the physician, after reviewing the clinical signs and symptoms, noting the pre- and posttransfusion reaction temperature readings, and the quantity of blood received by the patient, will determine the type of transfusion reaction sustained by the patient (see transfusion reaction type summary attached hereto). After instituting appropriate studies and/or treatment, the physician will complete the Blood Transfusion Reaction Report.
- c. If the physician decides there is a possibility of IHBTB, he should immediately draw a blood specimen in EDTA (purple cap, Vacutainer, #18 needle, 5 ml tube) with great care to avoid hemolysis and send this immediately to the blood bank as a "stat" specimen entitled "Possible Hemolytic Transfusion Reaction" along with data identifying the patient. At this point in the absence of laboratory data, if in the judgment of the physician there is an excellent clinical possibility of a hemolytic transfusion reaction, or if he has noticed grossly pink or red plasma, he may institute mannitol therapy in accordance with the directions attached hereto, while the laboratory data are being determined.
- d. Within a short time laboratory reports should be available which would indicate the concentration of the pre- and posttransfusion

plasma hemoglobin and the pre- and posttransfusion Coombs tests. Significantly elevated plasma hemoglobin levels above a normal value of 2-4 mg/100 ml and/or a significantly increased Coombs test are excellent evidence of IHBTB when coupled with appropriate clinical signs, symptoms, and history. Mannitol therapy should be instituted immediately (see directions attached hereto).

- e. IHBTB is accompanied probably in every case and in variable degree by a consumption coagulopathy. Because the IHBTB may be superimposed on any number of very different underlying diseases, appropriate coagulation studies should be done to determine whether the coagulopathy is: (1) primary activation of the coagulation system with a secondary fibrinolytic component; (2) primary activation of the fibrinolytic system; or (3) a coequal activation of both. Since treatment is quite different for each type of coagulopathy and fraught with disaster if treatment regimens are interchanged, the coagulopathy must be categorized. Heparin, intravenously, is the indicated treatment for category (1); epsilon amino caproic acid for category (2); and Trasylol with or without heparin would be acceptable for category (3). The clinician should consult with the pathologist to obtain a set of diagnostic and base line coagulation studies. In consumption coagulopathy *the basic pathophysiology is the intravascular conversion of plasma to serum. The therapeutic principle is the titration of the patient by intravenous, indicated drug to revert the serum back to plasma as guided by the response of individual parameters in a periodically repeated coagulation profile.* Because at present there are many coagulation technics from among which pathologists have personal preferences, the set of coagulation parameters for diagnosis and for guiding therapy is best left to the individual pathologist. As with other data in the context of the IHBTB, the results of the coagulation studies must be recorded on the Blood Transfusion Reaction Report.
4. If the blood in the blood transfusion container appears hemolyzed and/or unusually dark or blue-black, the possibility of bacterial contamination of the donor blood (and septicemia in the patient) must be considered. The laboratory will routinely obtain a gram stain and cultural studies on the plasma from the blood transfusion container. The gram stain report will be delivered to the physician shortly after the blood transfusion container is received by the laboratory. Cultural studies will of course be available a few days later, too late to be of value in the management of the jeopardized patient.
5. Consultation with the attending pathologist is invited at any time.

TYPES OF TRANSFUSION REACTIONS, THEIR RECOGNITION, AND MANAGEMENT

1. Pyrogenic: Any rise in excess of 1.5°F over the pretransfusion temperature level considered significant, but not diagnostic. May be due to platelet and/or leukocyte antibodies in some cases.

Laboratory Workup: None.

Treatment: Symptomatic.

2. Anaphylactic or Allergic Reaction: Manifested by rash, pruritus, and angioneurotic edema, swelling joints. Extreme cases (anaphylaxis) may have wheezing, dyspnea, and even sudden death.

Laboratory Workup: None.

Treatment: Allergic or Hypersensitivity:

Benadryl 50 mg orally or Phenergan 25 mg orally. In more severe cases Prednisone or Prednisolone 20-40 mg daily.

Anaphylactic:

Epinephrine 1:1000, 0.3-0.5 ml/q 5 minutes.

Benadryl 50 mg IV or comparable dose of other antihistamine.

Hydeltrasol 20-40 mg IM or IV daily or comparable other cortical steroid.

Levophed 4 ml and 1000 ml of 5 percent glucose and water at 1-4 ml per minute adjusted according to response for hypotension.

3. Incompatible Hemolytic Blood Transfusion Disease: This is a potentially lethal condition and rapid accurate diagnosis and expeditious treatment can save the patient. IHBTB is defined as that condition in which intravascular hemolysis of sensitized erythrocytes is so accelerated as to portend a possible fatality. Any of the signs and symptoms listed on the Blood Transfusion Reaction Report may be manifested in varying degrees and combinations.

Laboratory Workup: See instructions attached hereto.

Treatment: Mannitol and therapy for consumption coagulopathy (see instructions attached hereto).

4. Circulatory Overload Reactions: Signs and symptoms of acute congestive heart failure are present.

Laboratory Workup: None needed.

Treatment: Individualized "standard treatment for congestive heart failure."

5. Contaminated Blood and Septicemia: Clinical manifestations of septic shock are present. This usually occurs with the administration of the first 50-100 ml of blood. There is evidence of rapid pulse, lowered or absent blood pressure, pale sweaty appearance, severe chills, feeling of impending disaster, coma, convulsions, and even sudden death.

Laboratory Workup: All that will be immediately available will be a gram stain on plasma from the blood transfusion container.

Treatment: Management for septic shock including diagnosis and treatment for consumption coagulopathy.

6. Citrate Intoxication: Citrate intoxication is most likely in infants who have been managed with exchange transfusions, but also can be seen in adults with numerous transfusions which have been given closely together and adults with impaired liver function. It is characterized by muscle tremors, EKG changes (prolonged ST segment, prolonged QT segment, pulsus alternans, depression of T-wave), and shock.

Laboratory Workup: As requested by attending physician.

Treatment: Ten ml of 10 percent calcium gluconate for each two liters of citrated blood (recently transfused) is prophylactic.

7. Potassium Intoxication: Potassium intoxication is most likely to be seen in infants with exchange transfusions using bank blood from 9-21 days old. In adults, potassium intoxication may be seen in anuric patients who cannot safely tolerate increases in serum potassium levels.

Treatment: Consider use of glucose and insulin intravenously to depress the potassium level among other methods of lowering serum K level.

8. Other Sequelae:

- a. Thrombophlebitis: Thrombophlebitis is usually seen with transfusions which have extended for more than 8 hours continuously in the same venipuncture site.

- b. Air Embolism: Air embolism causes shock and dyspnea. Abnormal cardiac sounds are also auscultated. The treatment is to place the patient on the left side, head down, feet up position.

9. Late Sequelae:

- a. Transmission of disease: Malaria, serum hepatitis, syphilis, brucellosis.
- b. Transfusional hemosiderosis.
- c. Delayed isosensitization.

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ATTENTION: Anesthesiologists

POST AT:

1. Operating Room
2. Postanesthesia Recovery Room
3. Emergency Room
4. Laboratory (Blood Bank,
Chemistry, Bacteriology)

ROLE OF ANESTHESIOLOGIST IN MANAGEMENT OF BLOOD TRANSFUSION REACTION

A Blood Transfusion Reaction (BTR) is any adverse sign or symptom caused by blood or blood component transfusion and not related to the underlying disease of the patient manifested during or shortly after that transfusion.

An Incompatible Hemolytic Blood Transfusion Disease (IHBTB) is defined as that condition in which intravascular hemolysis of sensitized erythrocytes is so accelerated as to portend a possible fatality.

Early infusion of mannitol will save the lives of many patients with IHBTB. To test quickly for intravascular hemolysis, plasma is checked for pink or red color, a direct Coombs test is performed, and the plasma hemoglobin level is determined. Findings are compared with pretransfusion specimens, and if hemolysis is evident, mannitol is infused immediately. The shorter the interval between the offending transfusion and the use of mannitol, the more effective the treatment. To expedite therapy, all hospital stations where blood transfusions are given stock mannitol as an emergency drug with explicit directions for its use. Also, consumption coagulopathy must be anticipated and managed if IHBTB is present.

1. BTR, including IHBTB, is a disease and must be diagnosed and treated by a clinician with consultative support from the pathologist.
2. IHBTB in the anesthetized patient presents signs and symptoms somewhat different from those encountered by the clinician at the bedside of the conscious patient. In the anesthetized patient receiving an incompatible blood transfusion, the anesthesiologist will note:
 - a. A marked generalized bleeding and oozing at the operative site.

- b. Severe shock out of proportion to the degree of blood loss.
 - c. An appearance of bleeding and oozing from venipuncture sites which were previously dry.
3. If such observations are made, the anesthesiologist should proceed in accordance with the instructions issued for the guidance of the attending physician and the management of blood transfusion reactions (*quod vide*).
 4. If IHBTD is diagnosed and consumption coagulopathy is present, the coagulopathy can be treated during open surgery (see Rock *et al*, Transfusion 9: 57, 1969).

ATTENTION: Laboratory Personnel: POST AT:

Blood Bank	1. Blood Bank Laboratory
Chemistry	2. Chemistry Laboratory
Bacteriology	3. Bacteriology Laboratory

ROLE OF LABORATORY PERSONNEL IN MANAGEMENT OF BLOOD TRANSFUSION REACTION

1. It is the function of the laboratory to generate accurate, pertinent data and to report it rapidly to the responsible physician. If the data are abnormal or pathological, it must also be reported to the attending pathologist immediately.
2. When the blood transfusion donor container is returned to the blood bank, the blood bank personnel should check for agreement of all identification on data on the container, the pilot tube, and the patient. Any discrepancy must be reported to the attending pathologist "stat." The laboratory must generate and deliver data essential to the diagnosis of IHBT as indicated below.
 - a. Blood Bank: The blood bank must determine immediately the Coombs test and its intensity on the pre- and posttransfusion specimens of the patient's blood.
 - b. Chemistry Laboratory: The chemistry laboratory must determine the plasma hemoglobin level on the pre- and posttransfusion specimens. Urine specimens from the patient taken at the time of the transfusion reaction and 4 hours later will be received by the chemistry laboratory and held in the refrigerator. The pathologist will indicate whether quantitative hemoglobin and a specific gravity determination will be required.
 - c. Bacteriology Laboratory: The bacteriology laboratory will perform a gram stain on a centrifuged aliquot of plasma from the blood transfusion donor unit and also prepare appropriate inocula of the plasma from the blood donor container for cultural studies.
3. The laboratory reports the data from the Coombs test, the plasma hemoglobin, and the gram stain to the attending physician as rapidly as they are ready. Any abnormal values are also reported to the attending pathologist.
4. Data from the Coombs test, the plasma hemoglobin, the gram stain, and *ANY OTHER LABORATORY WORK DONE IN CONNECTION WITH THE*

*INVESTIGATION OF THE TRANSFUSION REACTION MUST BE RECORDED ON THE
BLOOD TRANSFUSION REACTION REPORT AS IT IS DETERMINED.*

5. The recheck procedure (the retyping and the recrossmatching workup) can be done at a later time during the working day in most instances. Results of this recheck procedure must also be recorded on the *Blood Transfusion Reaction Report form*. If the posttransfusion reaction specimens show a significant increase in the Coombs test and/or a significant increase in the plasma hemoglobin levels, the blood bank should automatically prepare a compatible blood transfusion unit, crossmatched with blood drawn from the patient after the transfusion reaction has occurred. This blood will remain in the blood bank on a standby basis in anticipation of need for the treatment of the jeopardized patient, according to the decision of the attending physician.
6. Deliver the Blood Transfusion Reaction Report form after all pertinent data from the initial investigation have been recorded thereon to the attending pathologist.

TABLE I

MANAGEMENT OF TRANSFUSION REACTIONS

Findings	Treatment
Urticaria only Fever, chills, etc. 1. Examine patient's blood for: intravascular hemolysis (plasma hgb.) extravascular hemolysis (Coombs test) 2. Examine donor plasma for bacteria	Intramuscular antihistamines, resume transfusion if controlled 1. Stop transfusion 2. If laboratory tests are negative treat with antipyretics and sedatives. With positive findings start prophylactic treatment as below
Shock, hemoglobinuria, oliguria, bleeding	1. Maintain blood pressure with vasopressor 2. Maintain urine flow over 100 ml./hr. a. Mannitol 25 grams intravenously b. Fluids 3. Replace specific deficits when indicate. a. Fresh plasma, platelet-rich b. Fresh frozen plasma c. Fresh whole blood, if necessary 4. Antibiotics and hydrocortisone for septic shock

TABLE II

Mannitol Infusion Treatment for Potentially Lethal Hemolytic Transfusion Reactions

Note: Mannitol is stocked at this nursing station as an emergency drug and is immediately available on request.

DIRECTIONS AND PROCEDURE OF MANNITOL TREATMENT*

Infuse intravenously 100 cc of 20 percent mannitol solution within a 15 minute period. This solution is available in 250 ml bottles at all nursing stations, including this one. This dose will initiate a diuresis of 1 to 3 ml of urine per minute in an adequately hydrated patient. The same dose may be repeated if urine flow drops below 100 ml per hour for any subsequent two hour period. Mannitol may be discontinued when the patient can maintain a urine flow of 100 ml per hour without its use. If diuresis does not occur, acute tubular necrosis may be presumed to exist and appropriate and immediate treatment for that condition is indicated.

COMMENT:

Time is of critical importance in treating incompatible hemolytic transfusion reactions. The degree of injury sustained by a patient is proportional to the time interval between the offending transfusion and the onset of mannitol treatment. Therefore, the sooner mannitol is administered the less severe is the injury.

If the history, physical findings and clinical course are such that a hemolytic transfusion reaction is suspected as highly probable, mannitol infusion should be started even prior to or concurrent with laboratory investigation, since under the conditions of use prescribed above, no direct adverse sequelae from the use of mannitol will occur. The gain increment of time difference in the interval between the use of mannitol and the laboratory determinations accrues to the advantage of the patient.

These explicit instructions for the use of mannitol are instantly available to the physician as is mannitol itself at each hospital station where blood transfusions are administered.

*From Postgrad. Med. 45: 84-89, 1969, based upon Barry, K. G. and W. H. Crosby, Transfusion, 3: 34-36, 1963.

The Blood Transfusion Reaction Report (Figs. 1a, b, c, d) serves multiple important purposes and when completed is a most valuable document. Initially, is used to guide the nurse's activities. Then it serves as a summary record of the salient clinical features as observed by the clinician at the time of the event. It routes and accompanies appropriate specimens to the laboratory. Data from the Coombs tests, the plasma hemoglobin tests, and the gram stain are recorded thereon.

If any of these determinations is abnormal, it is routed directly to the pathologist who may use it as a checklist for following the pertinent parameters in the jeopardized patient and for monitoring the response to therapy.

The form also incorporates data resulting from coagulation studies. The last and final function is an orderly summary record of the episode in sufficient detail to dissuade legal harassment.

In most instances only a very few entries will be made on this record. But, since it must be adaptable to the occasion of an IHBT, it serves as a most convenient prospective checklist for studies essential in the management and monitoring of the jeopardized patient.

PATIENT IDENTIFICATION

CLINICAL
DIAGNOSIS: _____

HOSPITAL
BLOOD UNIT
NUMBER _____

AMOUNT OF
BLOOD RECEIVED
BY PATIENT _____ ml.

ATTENTION: Nursing Staff

1. **STOP** transfusion immediately, leave needle in vein with slow saline drip.
2. **SUMMON** any available **PHYSICIAN** immediately.
3. Check for agreement, all identifying names, numbers, and letters on pilot tubes, transfusion unit, and patient's wrist tag.
4. Complete requisite data on this page.
5. Obtain an immediate post-transfusion urine specimen and a second post-transfusion urine specimen 4 hours later. Forward properly labeled specimens to laboratory.
6. Request physician to complete page 2 of this booklet.
7. Send to Blood Bank entire blood unit and this booklet with pages 1 and 2 completed.

	Pre-Reaction	Reaction	Post Reaction
Date			
Time	(Blood Started)		(Blood Stopped)
Temperature			
Blood Pressure			
Pulse Rate			

**PHYSICIAN'S EVALUATIONS
OF
CLINICAL MANIFESTATIONS (✓)**

Apprehension _____

Chilly Sensation _____

Severe Shaking Chill _____

Severe Low Back Pain _____

Pulmonary Infarct _____

Nausea _____

Arthralgia _____

Hives _____

Headache _____

Angina _____

Skin Changes _____

G. I. Hemorrhage _____

Dyspnea _____

Perspiration _____

**Oozing From Wound or
Venipuncture** _____

ADDITIONAL COMMENTS:

LABORATORY USE ONLY:

Date _____

Technician _____

Time Received _____

IDENTIFICATION DATA CORRECT? (✓) YES () NO ().

RE-CHECK OF TYPING

	Coombs		Cell Typing							Serum Typing			
	Direct	Indirect	A	B	A, B	Δ_0	R_{H_0}	rh'		A ₁	A ₂	B	O
Patient's Pre-reaction Blood Sample													
Patient's Post-reaction Blood Sample													
Pilot Tube - 1													
Pilot Tube - 2													
Donor Blood From Container													

RE-CHECK OF CROSMATCHES

	Saline	Coombs	Albumin	Immune Antibody: Anti _____			
				Titer	Day of Reaction	Day - 1	Day - 2
Patient's Pre-reaction Serum Sample							
Patient's Post-reaction Serum Sample							

BACTERIOLOGICAL STUDIES:

GRAM STAIN:

CULTURE:

BLOOD TESTS

	Pre-reaction	Day of Reaction	Day - 1	Day - 2	Day - 3	Day - 4	Day - 5
Hemoglobin							
Hematocrit							
Reticulocyte Count							
Serum/Plasma hemoglobin							
Bilirubin							
Methemalbumin							
Serum Haptoglobin							
<u>URINE TESTS</u>							
Free Hemoglobin (Quantitative)							
Free RBC							
RBC Casts							
Bile							
Albumin							
Daily Output							
Specific Gravity							

Figure 1c

CONSUMPTION COAGULOPATHY DATA

COMMENTS/CONCLUSIONS

MD

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Figure 1d

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A N N E X Q
CIVIL DISASTER AID - AN EXAMPLE

CIVIL DISASTER AID - AN EXAMPLE

Excerpts from the transcript of the Meeting for Review and Evaluation of Emergency Medical Services Following the October 30, 1972, Illinois Central Commuter Trains Accident, held under the auspices of the Chicago Hospital Council on November 13, 1972, AHA Building, Chicago, Illinois, are given below.

Quotes from the following are included:

Dr. H. Lawrence, Medical Director of Michael Reese Hospital and Medical Center

Mr. Robert Shakno, Vice President of Michael Reese Hospital and Medical Center

Mr. John McIntire, Associate Director of the University of Chicago Hospitals

Mr. Howard F. Cook, Executive Director of Chicago Hospital Council

Mr. C. Pepp, Deputy Chief of Police from the Chicago Police Department

Deputy Chief Pepp: "Another thing that entered my mind is - I wasn't actually on the scene at the hospital - that there was a call for blood that gave us a little traffic problem. I don't know the feasibility of it, but I throw it out for your consideration - if a need for blood could be taken care of at some hospital away from the emergency hospital, it would keep the avenues of transportation much more open. I don't know if you need the blood right there or if you can use the blood that's there and replenish it with blood from another hospital, but we ran into a little traffic problem because of the willingness of citizens to donate their blood.

"I referred to it as a call for blood. As soon as you called for blood, we got all kinds of traffic asking for directions to Michael Reese or where they could go to give blood."

Mr. Shakno: "All right. This was much earlier. We did get response then and officers were stationed around the campus and were able to control the inflow of traffic. So I think it comes back to a point made earlier - that traffic control in or near the scene and around the hospitals is critical. And it worked beautifully there once it got started; then the flow of traffic slowed down."

Mr. Cook: "In this particular instance, in regard to blood, it was suggested that in the future, if there is need for blood, there be a place for people to go away from the scene of that accident; and that would have been sufficient this time because you had enough on yourselves to care for quite some time. What people were looking forward to was that there was an afternoon and an evening as well as a morning. So I think we've got that suggestion on that type of thing."

Dr. Lawrence: "There are certain things I would like to say. One is that we did not issue a call for blood. And when I tried to find who did, the only thing I could identify was possibly the Chicago Metropolitan Blood Council. They issued a call without consulting the director of the blood bank, which, incidentally, for those people who may not be familiar with the campus, is about two and a half blocks from the emergency room. Nevertheless, because it's at a critical corner, Lake Park and 31st Street with a roadblock there, it did create long lines; and in fact, it created so many donors that, using all of our resources, we couldn't service the donors as fast as they could accumulate in the lineup. Here it's been pinpointed that there's a major problem in communications, and indeed it's been true not only now but in the past."

Mr. McIntire: "Just two things. One on the matter of blood. I'm glad Dr. Lawrence has made comments on the blood situation, because I wouldn't like to pass over that too quickly. We know that Michael Reese had this tremendous load and so we did get through to them and ask if we couldn't start drawing for them. We did. Then the helicopters came and we found that we were doing all we could do just to keep our own supply there for the needs of Billings Hospital. Michael Reese has really extensive blood drawing and blood banking facilities, and had the accident occurred somewhere else there may have been a big problem that we're not talking about today. Unless you're really prepared to cope - and I don't know who is - I'd be very wary of making announcements of the need for blood because blood donors come and you don't know where they come from."

Mr. Cook: "It's been pointed out there are forty-five hospitals that had blood drawing facilities, so that we're hardly ever going to have accidents that are going to put our blood drawing facilities out of order. In addition to that, we have a certain number of mobile units around town and we have a certain number of settlemental blood banks around town. We've probably got at least sixty or seventy locations where blood can be taken. Are any five or ten of those to offer us assistance, it can't hamper us too much."

Mr. McIntire: "When you do go into it, as we found, it isn't a problem of the blood donors. As we found out a couple of years ago, we needed technicians. I don't know how Michael Reese approached it, but we had to take people from among other laboratories. I'm just saying it's an issue, like transportation. Blood could have been an issue in this much more so than it was. The transport of blood had to be coordinated; it's just not something that's going to happen on its own."

"The only other point I'd mention is something that came up in New York City as a result of a terrible disaster that they had when an aircraft carrier caught fire. They had young men that were trapped right in the aircraft carrier. As a result of that disaster, everybody got together at a meeting like this and they outlined a plan where there would be one person in charge of the entire site. We've talked about medical hospital personnel, telephone company, Fire and Police, and I just wonder if we wouldn't want to talk about the need for an overall authority."

Excerpts from an Metropolitan Chicago Blood Council Memorandum to Administrators and Blood Bank Directors of Participating Hospitals and Blood Banks, November 7, 1972, are as follows:

At 9:15 A.M. on October 31, 1972, the MCBC contacted the Michael Reese Hospital Blood Bank to assess the transfusion requirements and available blood supplies for patients being extricated from the wreckage of the two Illinois Central trains which had collided at the 27th Street Station. At 9:30 A.M., Michael Reese expressed concern about its supplies of the following blood types: AN, BN, BP, and OP. No substantial surplus of these blood types had been reported to the Blood Information System during the previous week. At 9:45, with the agreement of Michael Reese, the MCBC contacted the City News Bureau, which, through a mutual agreement with the Chicago Hospital Council's Joint Committee on Hospital/News Media Relations, serves as a clearinghouse for emergency blood donor appeals.

An appeal for type-specific blood donors was initiated, and donors were requested to go directly to Michael Reese. (The Michael Reese Blood Center also serves Mercy, and Louise Burg Hospitals, and coordinated blood banking operations for these hospitals during the emergency.) The appeal included the statement that the hospitals had been able to supply blood to meet the needs of the initial wave of patients, but that many persons remained in the wreckage and the hospital was concerned that blood supplies of these specific types might be inadequate to meet their needs. This appeal was first carried by radio station WBBM at approximately 10:05 A.M., and was subsequently broadcast by other radio stations, including WIND, WGN, and WCFL. NBC-TV interrupted its regularly scheduled programming to broadcast the appeal.

At 11:10 A.M., the MCBC contacted Michael Reese, which reported that several hundred volunteer donors were at the hospital, and that "the situation was under control." At that time, the MCBC contacted the City News Bureau to cancel the emergency appeal. At the same time, a statement was issued suggesting that volunteer blood donors contact the hospital or blood bank nearest them to determine if supplies of blood were adequate. This information was carried on the City News Bureau wire approximately 11:15 A.M.

Throughout the period of emergency, the MCBC received calls from supplementary blood banks throughout the area offering back-up support to hospitals receiving patients from the IC disaster.

According to the Blood Information System records, 1,055 units of blood were collected from volunteer donors on October 31, and 573 units of blood were transfused, fractionated, outdated, or shipped to areas outside metropolitan Chicago. The Blood Information Operator is attempting to move the surplus blood to those hospitals in the area reporting shortages at this time.

PLAN TO PROVIDE BLOOD IN DISASTER SITUATIONS

OBJECTIVES

1. To provide adequate supplies of blood and blood components to disaster victims.
2. To provide blood and components, ready for transfusion use, as quickly as possible.
3. To provide blood and components with minimal disruption of the flow of traffic surrounding institutions providing emergency medical services to victims.
4. To maintain and replenish blood supplies to normal levels as quickly as possible.
5. To minimize blood surpluses generated during disasters and large-scale emergencies.

ESSENTIAL FUNCTIONS

1. Determine institutions receiving victims and assess status of blood and blood component supplies in receiving institutions.
2. Locate nearest available supplementary blood supplies available for transfusion use.
3. Arrange emergency transportation for supplementary supplies.
4. Arrange clearance for emergency vehicles at receiving institutions.
5. Maintain surveillance and repeat Steps 2 through 5, as necessary.
6. Assess status of blood supplies at supplementary institutions.

7. Initiate selective call for type-specific donors at supplementary and/or nonparticipating institutions.
8. Redistribute surpluses, if generated.

FUNCTIONAL RESPONSIBILITIES

1. Receiving institution reports immediately to the Blood Information Operator, as soon as notified that disaster patients are being dispatched, and reports status of blood supplies on hand.
2. Blood Information Operator polls hospitals and supplementary blood banks nearest receiving institutions for status reports and alerts MCBC office.
3. Receiving institution notifies Blood Information Operator if supplementary blood supplies are needed. Blood Information Operator locates supplementary supplies.
4. Supplementary supplier dispatches blood and/or components to receiving institution and advises Blood Information Operator of mode of transportation (fire, police, private auto, taxi, etc.).
5. Blood Information Operator reports mode of transportation to receiving institution.
6. Supplementary supplier determines need for replacement donations and advises MCBC. Selective donor recruitment by telephone initiated if necessary.
7. In the event of widespread shortages, media appeal for replacement donations initiated by MCBC following consultation with Blood Information Operator and participating institutions.
8. Blood Information Operator assists in restoration of inventory balances as required.

NEEDED FOR IMPLEMENTATION

1. Establishment of a Disaster Planning Committee to review this proposal and submit a final plan for promulgation.
2. Revision of Blood Information System data base to include in daily status reports all units of whole blood and packed red cells not on crossmatch.
3. Establishment of liaison with police, fire, civil defense, and other agencies involved in disaster planning to develop effective avenues of communication during disasters.

4. Preparation and distribution of windshield signs to identify vehicles transporting blood during disasters.

A N N E X R

**CIVIL DISASTER--LOD AIRPORT INCIDENT, ISRAEL
30 MAY 1972**

CIVIL DISASTER--LOD AIRPORT INCIDENT, ISRAEL*

30 May 1972

Transfusions given to 56 casualties in the emergency room and operating theater of the Sheba Medical Center on the night and following day of the Lod massacre:

<u>Product</u>	<u>Units</u>
Whole blood	48
Plasma	4
Hartman's solution	26
Glucose	21
Saline	7
Glucose saline	<u>30</u>
TOTAL	136

*Information furnished by Dr. A. Ben-David, Director, Israel Institute for Biological Research.

A N N E X S
MILITARY BLOOD BANKING
PITFALLS OF BLOOD GROUPING AND
PRETRANSFUSION TESTS

MILITARY BLOOD BANKING

PITFALLS

OF

BLOOD GROUPING

AND

PRE-TRANSFUSION TESTS

A MONOGRAPH

September 1968

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US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Major General Joe M. Blumberg, MC

Commanding

US ARMY MEDICAL RESEARCH LABORATORY

Colonel Ralph H. Forrester, MC

Commanding Officer and Director

PREFACE

Modern concepts of medical care demand an ever-increasing volume of blood safe for transfusion. In striving to meet this demand, one finds no less responsibility imposed upon the military than has been placed upon the civilian medical facility. Thus, the complex and interdependent series of procedures for pre-transfusion testing of blood evolved during the past two decades has become equally important to today's military and civilian blood banks alike.

Although there remain many voids in our knowledge, human error continues to be the major cause of untoward reaction to blood. Instances of human error, furthermore, seem more often the result of clerical than technical mistakes. The selection of a specific unit of blood for a given patient is a decision bearing absolute finality akin to no other laboratory procedure. There simply is no way to recover the wrong unit of blood once it has been infused in a recipient.

Because this responsibility for the selection of blood rests entirely upon laboratory personnel, it is our conviction that training and discipline in blood bank laboratory technique cannot be overemphasized. Through intensive training we can expect to fill some voids in knowledge and to minimize the chance of faulty judgment. Through incessant practice and experience we may expect to reduce the chance of clerical mistakes.

This compilation of the pitfalls in pre-transfusion testing of blood has been undertaken with the hope that it may help to minimize risks inherent in transfusion therapy. This monograph, which will be incorporated as one section of a workshop manual in Military Blood Banking, is intended for all personnel having technical or administrative responsibility to insure the safest possible blood banking practices in the military establishment.

Lt. Colonel Frank R. Camp, Jr. was Research Immunohematologist at Walter Reed Army Institute of Research, Washington, D. C., during the early stages of this monograph. He is currently Director, Blood Transfusion Division, US Army Medical Research Laboratory, Fort Knox, Kentucky.

During most of the preparation of this material, Colonel Frank R. Ellis was Clinical Pathologist, Wayne County General Hospital, Eloise, Michigan. He is currently Director, Southeastern Michigan Red Cross Blood Center, Detroit, Michigan.



Fort Knox, Ky.

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Lt. Colonel, MSC, USA



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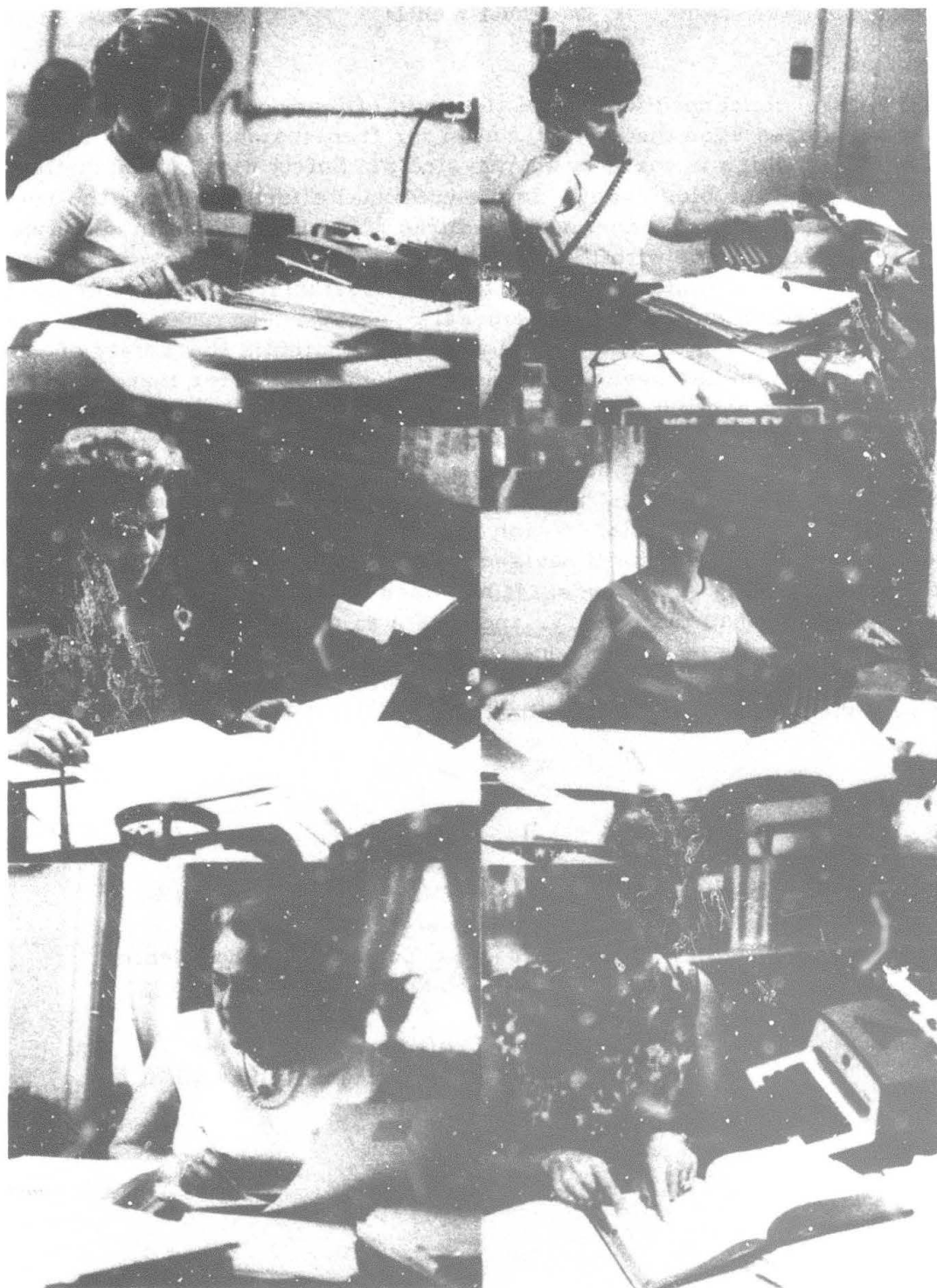
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FOREWORD

We are concerned with the safety and efficacy of blood transfusion. Efficacy hinges upon the patient's need for transfusion, and this is a determination which is made by the physician. Safety of the transfusion devolves upon the blood bank. In the continual effort to reduce the reaction rate, our blood banking procedures become more refined and numerous. Experience with these procedures inevitably uncovers unsuspected faults and foibles for which the transfusionist must remain alert. It is a dangerous and difficult business.

One of the Army's educational efforts to maintain the safety of blood transfusion has been an annual "Workshop" held for many years at Walter Reed and latterly at the US Army Medical Research Laboratory, Fort Knox. As a teaching aid in these workshops there was developed a pamphlet which details the shortcomings, the shortcuts, the faults, and tricks of the laboratory procedures used in blood banking. It was nicknamed "Pitfalls," which are traps for the unwary. Over the years, Pitfalls has been revised, improved, and expanded on the basis of continuing experience. It has been hammered out in the laboratory rather than the library, and it is a valuable tool, getting down to the very details of reagent quality and technician skill.

It is well to have good tools in a job where the cost of errors may be reckoned in terms of human life.

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NOTE: To avoid ambiguity, symbols for blood factors and their corresponding antibodies are printed in bold-face type.

PITFALLS OF BLOOD GROUPING TESTS

General Principles

Although some fifteen independent blood group systems have been discovered during the past sixty-five years, we will emphasize only those systems having importance in transfusion therapy and in the management of obstetrical patients and their newborn infants. Our concern over these specific problems arises from the knowledge that some of these blood group systems have attributes which may cause serious injury or death of patients who require the administration of blood. We also wish to avoid sensitizing female recipients with blood group antigens capable of causing erythroblastosis, if such recipients should later become pregnant.

As in all blood group systems, the scheme for designating the major A-B-O groups depends upon which antigen is present on the erythrocytes. Thus, the red cells of a specimen of group A blood contain the blood group antigen A, and cells of a specimen of group B blood contain the blood group antigen B. In a similar way, blood which is Rh-positive has erythrocytes which contain the antigen Rh₀(D). Red cells lacking the antigen Rh₀(D) are said to be Rh-negative.

The presence of any given antigen on an erythrocyte may be demonstrated by the use of a blood grouping serum containing the antibody specific for that particular antigen. Under appropriate testing conditions, for example, an anti-A blood grouping serum will agglutinate red blood cells containing the antigen A. Because this agglutination can be seen, we recognize that some sort of reaction has occurred. We, therefore, infer that the anti-A antibody in the serum has attached itself to the A antigen on these cells and that agglutination resulted by this means. When cells containing only the antigen B are exposed to an anti-A serum, no agglutination will occur. Under these circumstances, we must conclude that there is no antigen A on group B erythrocytes.

The testing of unknown cells with sera of known antibody content is referred to as direct or cell grouping. A positive cell grouping test indicates the presence of the antigen, and is recognized because agglutination occurs. Failure of an antiserum to agglutinate a sample indicates the absence of that antigen.

The results of red cell A-B-O grouping tests may be verified by determining which antibodies are present in the serum of the blood sample. This confirmation technique is known as reverse or serum

grouping. It involves the use of three separate samples of erythrocytes known to contain antigens A, B, and O for testing the unknown serums. If such an unknown serum agglutinates B but neither A nor O cells, one learns that anti-B antibody is present in that serum. There is an invariable reciprocal relationship between the antigens A and B and their respective agglutinins, anti-A and anti-B. For this reason, we expect to find anti-A antibody in the serum of group B persons, anti-B antibody in the serum of group A persons, both anti-A and anti-B antibodies in the serum of group O people, and neither anti-A nor anti-B antibodies in the serum of persons having group AB blood. Existence of this reciprocal antigen-antibody relationship permits reinforcement or proof of the validity of the results of the cell grouping tests. Instead of seeking the identity of an unknown antigen by use of a serum of known antibody specificity as was described for the cell grouping technique, reverse or serum grouping techniques seek the identity of any antibody that might be present in an unknown serum through the use of cells of known antigenic components. Now, the occurrence of agglutination indicates the presence of an antibody in the unknown serum which is specific for the antigen on the test cells. It follows logically, then, that the absence of agglutination tells us there is no antibody in the unknown serum for the antigen on that specific test cell. It is appropriate to mention that agglutinates resulting from serum grouping tests may be much less intense than those observed with cell grouping sera. Reasons for these differences in intensity of agglutination will be discussed below (potency of the reagent, page 16). We have no similar built-in opportunity to verify results of cell grouping tests in any of the other blood group systems because the A-B-O system alone has naturally occurring isoantibodies, always reciprocal to the antigens present on the cells. While we are quick to acknowledge reports of "naturally occurring" antibodies in the Rh-Hr, Kell, I, Ss, Wr^a, and perhaps other blood group systems, we prefer to stand on the conservative hypothesis stated above. Our reasons for this position result in part from the rarity frequency of such examples as compared with the vast numbers of experiences to the contrary side, and more importantly, with our inability to totally explain the existence of "natural" isoagglutinins even in the A-B-O system itself. As is the case so often in the practical application of serologic techniques, inconsistency may result from the inescapable need for arbitrary definition of terms. It may thus be argued that even the antibodies consistently found in the A-B-O system are, in fact, themselves immune and the result of environmental immunization of some kind. Experience has shown, nonetheless, regular presence of antibody in the A-B-O system which can and should be used

for serum grouping confirmation tests. All antibodies specific for antigens of all of the other blood group systems are irregular, and may be regarded as de facto evidence of prior immunization. The more commonly encountered irregular agglutinins are in the Rh-Hr, Kell, Duffy, or Kidd systems. Irregular antibodies have also been found for each of the remaining blood group systems including the low incidence or "private" blood group antigens that have so far been established. As a matter of interest, it is precisely in this way that the existence of a new system may first be suspected, and ultimately be proved. Table I shows the serological reactions upon which the A-B-O system is based.

Table I

Serologic Classification of A-B-O System

Red Cell Grouping				Serum Grouping				Phenotype
Reactions with known sera			Antigen present on cells	Reactions with known cells			Antibody present in serum	
Anti-A	Anti-B	Anti-A,B		A	B	O		
+	-	+	A	-	+	-	anti-B	A
-	+	+	B	+	-	-	anti-A	B
+	+	+	AB		-	-	none	AB
-	-	-	O	+	+	-	anti-A,B	O

Symbol + indicates agglutination; - no agglutination.

Blood Grouping Reagents

Blood grouping reagents are sera. They are usually, but not always, of human origin and contain agglutinating antibodies capable of detecting specific blood group antigens. They are labelled according to the antibody they contain, e. g., "anti-A serum," which will agglutinate only cells bearing the blood group antigen A.

Reliable serologic results can be expected only if potent specific antisera are available, the proper technique is employed in their use,

and they have been protected by proper storage conditions. Proper technique includes not only methods of mixing serum and cells, temperature and interval of incubation, and reading of results, but also the less obvious points of cleanliness of glassware, choice of control cells, and care to avoid contamination of antisera and test cells alike.

Subgroups of A

Blood possessing antigen A can be classified into two main groups. The eighty per cent of A bloods that are agglutinated by a subgrouping serum (anti- A_1) are classified as A_1 (or A_1B). Those failing to be agglutinated by this anti- A_1 serum are classified as A_2 (or A_2B) or weaker, A_3 , A_4 , etc. These latter groups may be so weak as to even fail to be agglutinated by potent anti-A serums. These have been classified as A_3 , A_4 , etc.

About one per cent of A_2 and one quarter of A_2B individuals have the irregular antibody, anti- A_1 , in their serum. A proportion of A_1 and A_1B persons have the irregular antibody anti-H in their serum. These peculiarities are worthy of note, and can be a pitfall to the uninitiated. An excellent description of the subgroups of A weaker than A_2 (A_3 , A_4 , A_x) is presented in the thesis by Arne Gammelgaard.

Standards of Blood Grouping Reagents

The Division of Biologics Standards, National Institutes of Health, has established minimum requirements for the manufacture and labeling of all of these reagents offered for sale in interstate commerce. Applying to all such materials, antisera must be sterile, non-turbid, have proper specificity, potency and avidity, and be free of hemolysins, autoagglutinins and atypical antibodies. The avidity of a testing serum is a measure of its ability to agglutinate rapidly and completely. While the criteria for test cells or other antisera will differ in detail, Table II defines minimal requirements for anti-A and anti-B grouping sera.

Table II

NIH Minimum Requirements
for
Anti-**A** and Anti-**B** Grouping Serum

Serum	Test Cells	Titer	Avidity* in Seconds
anti- A	A ₁	256	15
	A ₂	128	30
	A ₁ B	128	30
	A ₂ B	64	45
anti- B	B	256	15

*Term used to define the recognizable beginning of agglutination.

Reagent Red Blood Cells (Human)

Over the past few years almost all of the commercial supply sources have introduced products known as Reagent Red Blood Cells (Human). These group O cells are from separate donors or prepared as pools of several donors, depending upon the use intended for the product. For the detection of irregular antibodies, pooled reagent red blood cells are offered commercially for routine screening of sera, although the use of separate cells has been demonstrated to be a more satisfactory technique. For the identification of irregular antibodies, sets of eight or more selected donor cells bottled separately must be used, and are sold as "panels."

The National Institutes of Health (NIH) has moved cautiously in defining minimum standards for this class of blood bank reagents because of the complex problems these products have posed. While the use of preservatives is permitted, the manufacturer must demonstrate the absence of an adverse effect on reactivity and specificity of the product during the dating period. Labels or package inserts must include the blood group factors present on the cells, the concentration of unhemolyzed cells, the nature of any additives, and a

cautionary statement that the product may show diminished reactivity during the dating period. Where pools are offered for use, there shall be no less than 30 per cent of the cells in the pool bearing the factors stated on the labels. The usual requirements of sterility, restricted range of temperature in storage, and holding of reference samples from each lot released also apply to these products.

While the use of Reagent Red Blood Cells (Human) can be extremely helpful, their failures can also be legion. The more sophisticated blood banks will find that selection of donors regularly available to them, especially for use as screening cells, will often provide more satisfactory results than purchasing such products. The reasons for this statement are that cells need not be stored as long (storage deterioration in reactivity is a prime pitfall) and that the use of two separate cells is infinitely more satisfactory than even the same two cells will be in a fifty-fifty pool. Suspensions of Reagent Red Blood Cells (Human) in which only 30 per cent of the cells in the pool carry the blood factors reduces the number of antibodies detected even further.

With panel products for identification of irregular antibodies, the solution is far less simple. Storage deterioration of reactivity is as equally severe as in the screening products, although these are not pooled, but single donor cells. To assemble a set of cells containing certain antigens in specific combinations can tax the ingenuity of anyone. The ability to market such sets on a continuing basis even with a twenty-one-day period of dating has almost defied solution. To assure reliable results, the laboratory should have at least three different panels on automatic issue, scheduled so that one fresh panel arrives each week. Even so, not all antibodies encountered can be precisely defined with these products.

It, therefore, becomes necessary quite often to enlist the assistance of reference laboratories for solving such problems.

Sources of Blood Grouping Reagents

Anti-**A*** and anti-**B** blood grouping sera are obtained from humans immunized either through pregnancy, transfusion, or as volunteers injected with blood group specific substances.

*To avoid ambiguity, symbols for blood factors and their corresponding antibodies are printed in bold-face type.

Anti-A, B (group O) serum is also obtained from immunized human donors. Group O sera are selected according to their ability to detect very weak A antigens. Reaction against A and B cells is stronger with this reagent than with anti-A or anti-B, possibly due to a cross-reacting antibody (sometimes called anti-C). It is especially useful in detection of subgroups of A weaker than A₂. Its use is additionally recommended in routine A-B-O grouping as a predictable control. It should, of course, agglutinate all cells except those of group O.

Absorbed anti-A (anti-A₁) serum is prepared from serum of selected group B humans. Prior to processing, these sera contain alpha antibody (agglutinates both A₁ and A₂ cells) and alpha₁ antibody (agglutinates only A₁ cells). The alpha component is removed by absorption with A₂ cells leaving the alpha₁ component behind. The processed serum will agglutinate A₁ and A₁B but neither A₂ nor A₂B cells; hence, it is labelled "anti-A₁."

Red cells of A₁, A₂, B, and O specificity may be used as reagents for serum grouping tests. These may be selected within the laboratory using them, or purchased from commercial sources. Irrespective of source, working saline suspensions must be made fresh at least once each day and protected by refrigeration at 4°C when not in actual use. The group O cell will normally not be agglutinated unless some unusual antibody is present. It thus serves as a predictable control analogous to the use of group O serum in cell grouping procedures.

Another source of blood grouping reagents is a class known as Lectins. One of this group of saline extracts of seeds has anti-A specificity, and it can, by proper (but arbitrary) adjustment, be made selectively to agglutinate A₁ and A₁B cells, but not red cells containing the antigen A₂. Although lima bean extracts also possess A₁ specificity, extracts of Dolichos biflorus are commercially available under the label "anti-A₁ Lectin." Lectins are believed to show blood group specificity by a mechanism unlike that of antigen-antibody systems containing protein. In addition, they are adjusted by dilution to sharpen their specific response (especially Dolichos anti-A₁). For these reasons, Lectins may provide results that fail to agree completely when compared with absorbed anti-A sera in subgrouping specimens of group A blood.

Anti-M and anti-N sera are obtained either from humans, from immunized rabbits, or as Lectins (Vicia graminea with anti-N).

Anti-rhesus typing sera obtained from immunized humans are available in two general varieties. Sera containing principally incomplete or albumin-active anti-Rh₀(D) antibody are most common,

and they are labelled "for slide or rapid tube test" along with the specific antibody they contain. They may also have anti-Rh₀ (D) plus anti-rh' (C); anti-Rh₀ (D) plus anti-rh'' (E); or anti-Rh₀ (D) plus anti-rh' (C) plus anti-rh'' (E) activity. When used according to package instructions, they will detect only the activity noted on their labels. In short supply are saline-active (or complete) anti-Rh₀ (D) sera which are to be used only with saline-suspended erythrocytes for test tube tests. Such sera include in their label "for saline tube test only." Anti-hr' (c), anti-hr'' (e) and the rh' variant, anti-rh^w (C^w) are also available as either "slide or rapid tube test" or "saline tube test" reagents. The need to distinguish between incomplete and complete varieties of antisera arises from the necessity to employ different cell suspending media, incubation periods, and glassware (slide versus test tube) in order to obtain valid results. As noted above for anti-A and anti-B grouping sera, the NIH has defined minimally acceptable titers for anti-Rh typing serum to be 32 units, for both saline and blocking type sera. Standards have also been established for avidity, a term designating the recognizable beginning of agglutination which must occur within 60 seconds. Sera must be specific for the antigens stated on the label, sterile, and free from turbidity and excessive hemoglobin content as well.

Antiserum for any blood group antigen may be obtained from humans who, lacking a given antigen on their erythrocytes, have been immunized with red cells bearing the foreign antigen.

Anti-human globulin (Coombs') serum is a valuable reagent available only from a species other than man which is capable of forming an antibody against human serum protein. It is used to detect the presence of an incomplete or blocking antibody attached to, but incapable by itself of agglutinating, erythrocytes. It is most often made in rabbits or goats and, as for the blood grouping sera, must also be manufactured to minimum standards defined by the NIH. Antiglobulin serum is mentioned here simply in the interest of completeness and because it is required in connection with a number of blood grouping sera, including some antigens in the Rh-Hr system. A later section is devoted entirely to pitfalls involving this reagent (see page 20).

Technique

All blood grouping reagents are packaged with a circular of instructions provided by the manufacturer. These instructions include recommendations for obtaining optimal results with the particular antiserum. Inasmuch as some change is inevitable from lot to lot of

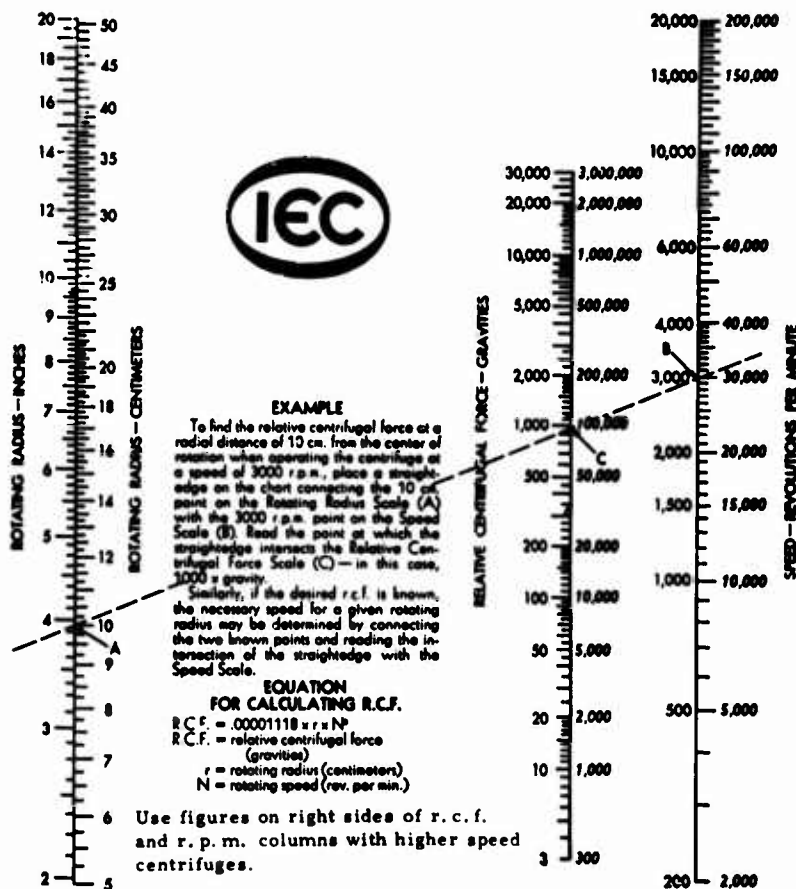
almost any antiserum, the manufacturer may find it necessary to alter the instructions periodically in order to assure accurate results. It is, therefore, necessary to read the instructions and to ascertain that no change has been introduced as new lot numbers are received. Use of recommended concentration of red cells, attention to specified proportions of cells and antiserum, care in incubation at the proper temperature for the designated time interval, and employment of proper centrifugation where required will assure accurate results for any given serum. If the package insert with a particular antiserum cautions against centrifugation, the addition of this procedure is apt to introduce a proportion of false-positive results.

Probably the least well controlled maneuver of all is centrifugation. Package instruction sheets, until very recently, uniformly stated the requirements for centrifugation in revolutions per minute (rpm) for a certain period of time. Owing to variation in the radii of centrifuge heads and speeds attained from one piece of apparatus to another, unbelievable differences in relative centrifugal force (rcf) have resulted. This variable can be standardized only when manufacturers specify the desired rcf, and laboratories operate their equipment to achieve these forces. The International Equipment Company, Needham, Massachusetts, has made available a nomograph (Fig. 1) from which rcf can be computed simply by measuring the radius of the head and the speed of the centrifuge. The rcf is expressed as a whole number times the force of gravity, e. g., $2000 \times g$. All blood grouping reagents currently being purchased for the Armed Services now require the rcf values, as well as rpm values for a stated time, be included in package circulars where centrifugation is required. Periodic calibration of centrifuges as a part of quality control within the blood bank laboratory is highly recommended.

Only the practice-acquired personal experience of a technician can minimize variables in reading results. The button of red blood cells must be resuspended carefully from the bottom of the tube. Read as you resuspend! Delicate agglutinates (as in the serum grouping part of A-B-O testing, or evaluation of anti-Rh antibodies) can be shaken out without one ever suspecting that they had been there. With increasing experience, most technicians will indicate relative strengths of serologic reactions by assigning one to four plus symbols on their protocol sheets, which we wholeheartedly recommend, for indicating weak to complete agglutination.

INTERNATIONAL CENTRIFUGES

NOMOGRAPH FOR COMPUTING RELATIVE CENTRIFUGAL FORCE



INTERNATIONAL EQUIPMENT COMPANY

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Fig. 1. Regular and frequent calibration of centrifuges not only equates an important variable in quality control, but provides a basis for more meaningful comparisons of data between different laboratories.

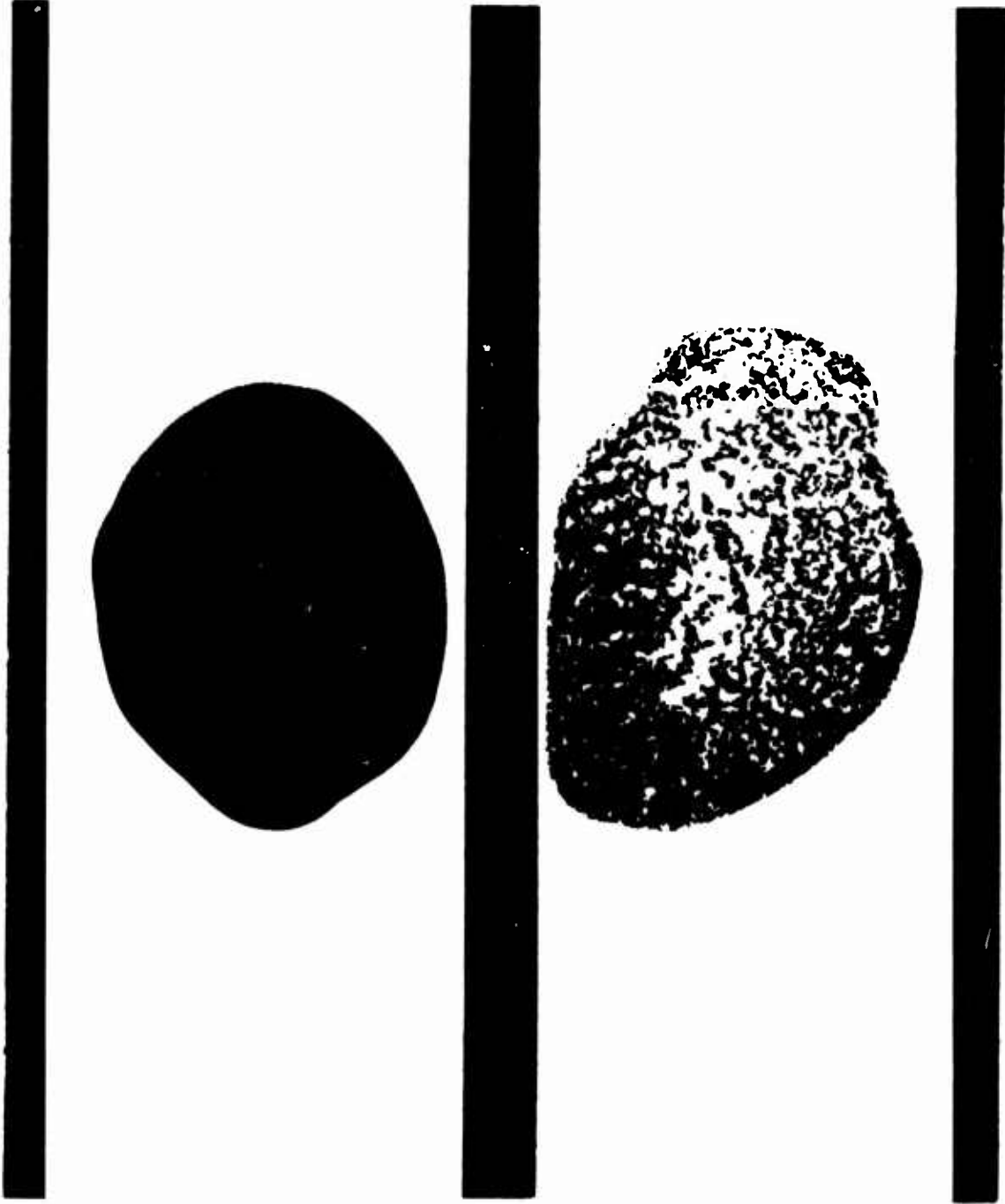


Plate 1. Photograph of slide tests showing negative on left and positive reaction on the right.

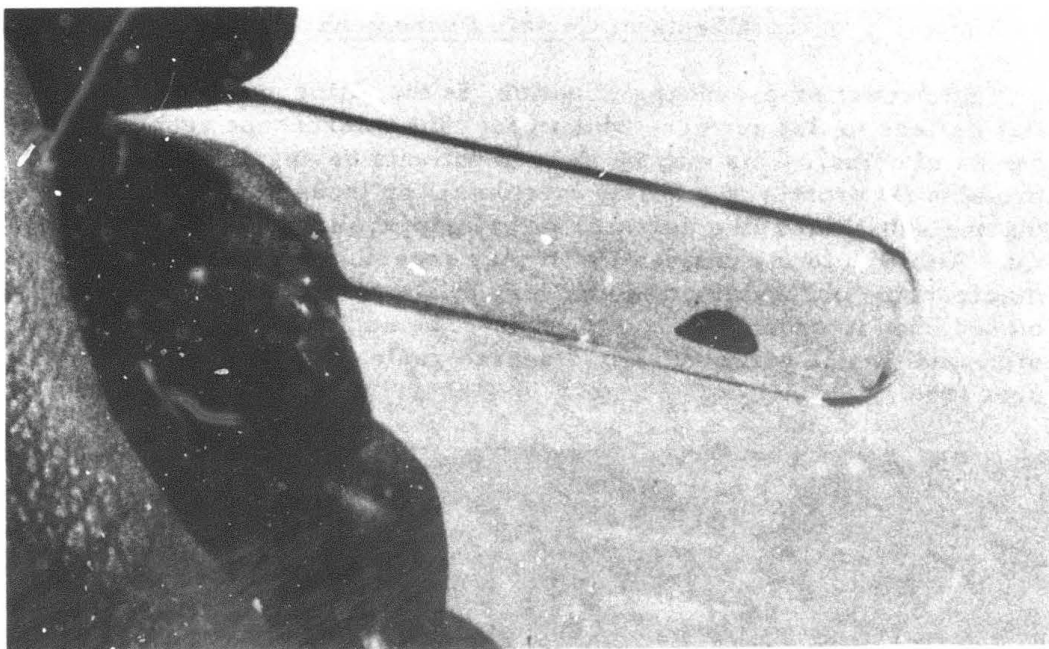


Plate 2. Tube tests showing ++++ (top) and +++ reactions (bottom).

Effects of Certain Phenomena

Rouleaux, or pseudoagglutination, is the piling up of red cells, flat surface to flat surface, which under the microscope resemble stacks of coins. This may be seen in patients having altered serum proteins (M protein of multiple myeloma), or those having received plasma substitutes like dextran, polyvinylpyrrolidone (PVP) or gelatin. Although to the unaided eye it may resemble agglutination (and macroscopic observation may be very misleading), its nature is disclosed upon microscopic examination. The addition of several drops of normal saline will generally disperse rouleaux, but has no effect upon true agglutination.

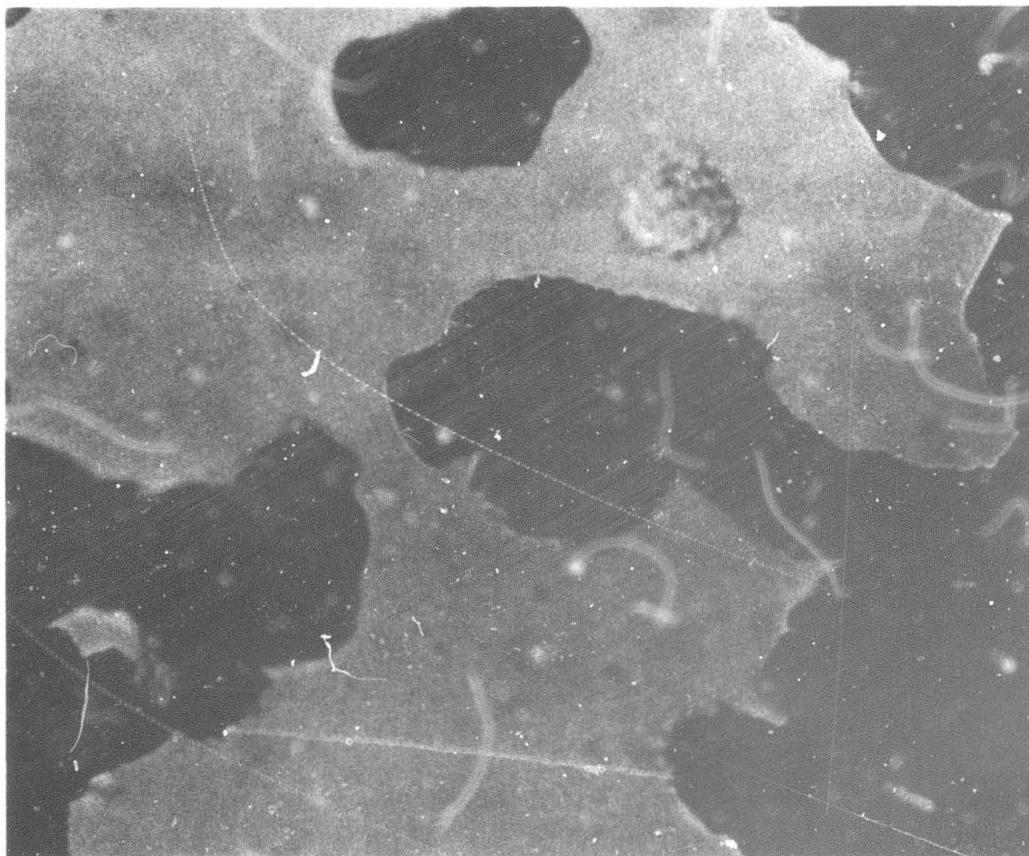


Plate 3. Photomicrograph of a 2% suspension of red cells in myeloma serum which produces marked rouleaux (X500).

Factors Influencing Agglutination per se

Red blood cells must be suspended in saline rather than a high-protein medium if being tested with a saline-active antiserum. To ignore the requirements of a given antiserum in this respect is to invite aberrant results.

A serum containing an incomplete antibody may be capable only of coating and not agglutinating its specific antigen. This fact can best be determined by use of the indirect antiglobulin (Coombs') technique. It is also possible that a saline antibody may be made incapable of producing crisp agglutination if the test cells are suspended in high protein or other macromolecular media.

Some blood group antigens, notably the weak variant of Rh₀(D), Rh₀(D^u), require use of the Coombs' antiglobulin technique for consistent detection. Antisera capable of detecting the antigens for Kell, Duffy, and Cellano almost always also require antiglobulin technique for agglutination to occur.

Some blood group antigens, especially Lewis, may require complement for detection. Use of heat-inactivated or long-stored serum would produce erratic results because complement deteriorates under either of these circumstances.

Natural antibodies, especially anti-A and anti-B, are usually most reactive in the cold. When performing serum grouping tests, for example, such antibodies might fail to react at all following prolonged incubation at 37°C. In contrast, "immune" antibodies are most reactive at 37°C. Thus, reactivity of an anti-Rh antibody might be undetected following incubation either at room temperature or 4°C.

When released for distribution, all lots of commercial sera, for which standards have been defined, have had NIH evaluation. Sera for which no standards have been defined are also available for sale or free distribution. These generally are for blood group systems not crucial to the operation of a transfusion service and are not so well monitored by NIH. Improper storage (especially without refrigeration) permits deterioration by loss of titer, avidity, or even specificity. The latter may be altered during storage even at the proper temperature by the slow reappearance of unwanted antibodies removed either by absorption or neutralization during the manufacturing process. Alteration in pH or electrolyte concentration may result from reconstituting a lyophilized serum differently than according to instructions, and such alteration may adversely affect the ability of a serum to react properly. Contamination by saprophytic microorganisms can alter a serum by making it nonspecific.

The immediately foregoing comments concern commercial typing sera. Antibodies found in random, unknown specimens also serve admirably at times as reference materials and as typing sera as well. They differ from commercially processed typing sera, however, in at least two important respects: They are almost always lower in titer, and they usually are of lower avidity. These two differences explain the delicate agglutinates and the risk of failing to detect agglutination in serum grouping tests referred to briefly on page 3 and above. Bear in mind that the same pitfalls enumerated for commercial sera may be encountered in sera you find and save for your own future use.

Hemolysis, or the rupture of red blood cells, may result from many causes. It is not infrequently associated with specific antigen-antibody reactions and is most often encountered in serum tests for A-B-O blood groups. Many immune isoantisera, in addition to producing agglutination, may also be capable of producing hemolysis of the erythrocytes. Complement, present in fresh serum, is necessary for hemolysis to occur in vitro. The significance of hemolysis is twofold. First, it is substantive evidence that an antigen-antibody reaction has occurred, and though often overlooked, has as much significance as solid clump agglutination. Second, pertaining to cross-match tests, hemolysin MUST be recognized in order that a unit of blood containing the antibody producing this hemolysis will not be transfused to a recipient possessing the susceptible antigen. Hemolysis may also result from glassware having soap or chemical residue, excessive temperature of a water bath, bacterial contamination, exposure of cells to freezing, improper salt concentration in homemade saline, excessive age of stored cells, or from inherited chemical or metabolic abnormalities of the red cells.

Cold agglutinins are antibodies usually without blood group antigen specificity which agglutinate red cells most intensely at temperatures near 4°C. This reaction is not infrequently seen with sera of patients having primary atypical pneumonia. Owing to their generally non-specific nature, cold agglutinins may cause confusion in A-B-O serum grouping as well as in the major crossmatching test. Since the effect of nonspecific cold agglutinins is reversible, cells agglutinated at 4°C will, upon being warmed briefly at 37°C, disperse into a smooth suspension. Recooling the mixture causes the agglutination to reappear. It is this phenomenon that permits identification of a nonspecific cold agglutinin with confidence. Once identified, cold agglutinins can be removed from a person's serum by incubation of the serum with its own cells at 4°C or in a chipped ice bath (ca. 0-1°C) for one or two hours. It is necessary to maintain the lower temperature even during centrifugation while the serum is removed from the

clot. By the same token, the nonspecific cold agglutinin may be eluted from a person's red cells by incubation of his serum at 37°C, and by maintaining this temperature when separating cells and serum. Specific antibodies in the I, M-N, P, and A-B-H systems usually are optimally active at 4°C. Almost all adults carry the I antigen on their red cells. Cells of the newborn show very little I antigen activity. In crossmatch problems due to cold agglutinins, anti-I specificity can be determined if cord cells known to be free of antigen I fail to react at 4°C.

Panagglutinins, which may be encountered in sera contaminated by bacterial growth, have the property of agglutinating all human red cells, irrespective of their antigenic composition. Panagglutinable erythrocytes may result from bacteriogenic cause, as with the Hübener-Thomsen-Friedenreich phenomenon, or from nonbacteriogenic causes as seen in acquired hemolytic anemia, which will be discussed later.

Bacteriogenic type panagglutination probably results from the action of some bacterial enzyme on the red cell surface, which renders them agglutinable by all normal adult sera; again, without regard for specific antigenic composition. Agglutination is believed to result from anti-T, an antibody which is active at room temperature and found in all normal sera except those of the newborn.

Nonbacteriogenic type panagglutinable erythrocytes are more often reactive at 37°C than at room temperature, although many specimens may fail to demonstrate this temperature differential. A somewhat more consistent point in differentiating the two is by the anti-human globulin (Coombs') reaction. Both the direct and indirect Coombs' tests are negative at 37°C in the bacteriogenic type, while these tests are usually positive in specimens of the nonbacteriogenic type.

Polyagglutinable erythrocytes differ from panagglutinable red cells in a subtle and perhaps not really fundamental way. These cells may be agglutinated by as many as 90 per cent of normal adult sera which are A-B-O group-specific, but they are not agglutinated by their own serum as is the case with panagglutinable specimens. Polyagglutinability has been seen most often in patients during, or for some limited period of time following, severe viral or bacterial infection, but it has been observed in apparently healthy persons as well. Although transient in nature, the reactions occur at lower temperatures, the indirect Coombs' test is negative, and it may well be the result of in vivo activation of the T antigen. These specimens are free of culturable bacteria, whereas a variety of microorganisms have been recovered from panagglutinating sera.

Autoagglutinins are most often encountered in patients with acquired hemolytic anemia. They rarely can be shown to have blood group antigen specificity. Depending upon whether they are most reactive at 4° or 37°C, they may be classified as being of the cold or warm variety. Irrespective of optimal temperature, they usually are also active at room temperature. They not only interfere with blood grouping tests, but also adversely affect tests for compatibility as well. The direct antiglobulin (Coombs') test is not infrequently positive in these patients because the autoagglutinin in the serum coats the patient's own cells. The indirect antiglobulin test may be employed to demonstrate the autoagglutinin in the serum of these patients. From what has been said earlier, it can be inferred that some autoagglutinins may behave also as panagglutinins.

When blood group specificity can be shown in cases of acquired hemolytic anemia, Rh-Hr specificity may be assumed if compatibility can be demonstrated with Rh-null (---) cells. Use of elution techniques would precisely define such things as anti-Hr^(e) antibody existing in a patient possessing this antigen. It is well to realize that a host of other causes of autoimmune hemolytic anemia has been suggested. Among drugs only recently associated with such phenomena is the hypotensive, methyldopa, or Aldomet®[®], which appears to produce a direct antiglobulin reaction in some patients. Thus far, this drug has induced a pure gamma-type response, while others (phenacetin or p-aminosalicylic acid) have caused non-gamma-type antiglobulin reactions. Thus, not only may these antibodies be classified according to optimal temperature, but with respect to the specific kind of antiglobulin serum which can detect them as well. The so-called gamma-type antiglobulin serum is made from essentially pure gamma globulin, while the non-gamma-type serum is prepared from globulins other than gamma.

Among the causes of hemolytic anemia unrelated to the appearance of antibodies but manifested by idiosyncrasy to drugs is so-called primaquine-sensitive hemolytic anemia. These patients have an inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase.

Irregular isoantibodies may be found in any of the blood group systems. Thus, anti-H found in the serum of A₁ and A₁B persons is unexpected (or irregular) and so is anti-A₁ in persons of A₂ or A₂B phenotypes. Unless untoward reaction to transfusion has occurred in group A or AB recipients, we ignore the subgroups of A in the operation of a transfusion service. The subgroups of A may, however, assume prime importance in serologic tests of disputed

paternity, in forensic immuno-hematology, and in resolving discrepancies within the A-B-O groups when processing donor or recipient samples. Confusion from irregular anti-A₁ antibodies may be greater in samples of subgroups weaker than A₂ (A₃, A₄, etc.), particularly when such weak antigens are paired as A₂B phenotypes owing to the high probability that the A antigen may be missed completely. Such a sample could, therefore, be incorrectly classified as group B.

The presence of more than one antibody in an unknown serum often serves to demoralize the inexperienced or inadequately trained laboratory worker. Thus, an already sensitized Rh-negative patient can be expected to have an anti-Rh₀ antibody in addition to the naturally occurring A-B-O antibodies. If the A, B, and O cells employed for serum grouping tests contain the antigen Rh₀(D), for example, the unknown serum may agglutinate all three test cells if it contained saline room temperature active anti-Rh₀(D) in addition to anti-A and/or anti-B antibodies. It is, therefore, essential that the antigenic composition of all cells selected for serum grouping be known, and that these cells be selected in a way capable of sorting out the complex sera described.

Peculiarities in Testing the Blood of Newborn Infants

The antigens of cord blood are often much less reactive than blood from older persons. As a result, agglutinates are more easily shaken out, and false-negative direct grouping tests may be obtained. Serum grouping tests of newborn samples may not confirm cell grouping results. When a discrepancy is found in such a sample, it is necessary to know the blood group of the mother, and to remember that most, if not all, antibody in the newborn serum is of maternal origin.

False-positive cell grouping tests on cord samples may result from contamination of the specimen with Wharton's jelly. When a disproportionate number of babies appear to be group AB, it would be well to review the technique of obtaining cord blood. It is exceedingly difficult to wash such cells free of this contaminant. Collection of samples without "milking" the umbilical cord will ordinarily avoid interference from Wharton's jelly.

It occasionally happens that sufficient maternal incomplete anti-Rh₀(D) antibody may be present in an Rh-positive infant to fully coat the baby's cells. When such cells are tested with anti-Rh₀(D) sera, they may fail to be agglutinated, and thus be reported erroneously

to be Rh-negative. Such an error in the laboratory may lull the clinician into a false sense of security, and compromise the chance of survival of an erythroblastotic baby. This infant's cells will invariably show a direct positive antiglobulin test, and its true Rh designation may be extremely difficult to establish by cell grouping techniques. Elution of the coating or blocking antibody from the baby's cells and demonstration of anti-Rh₀ (D) specificity in the eluate proves the cause of the aberrant result. In such infants, retesting after a few weeks will establish the proper Rh type for the record.

Control of Reagents

The use of positive and negative control cells in blood group serology is the sine qua non of good procedure. Where it is possible to use the same donors over prolonged periods of time, successive lots of sera can be used with even greater confidence. It is imperative that these cells be tested for antigens of other systems in addition to those in the system for which they are elected. Thus, when screening unknown serums for irregular antibodies, the use of group O cells will avoid agglutination by A-B-O antibodies, and will serve to alert the technician to possible irregular agglutinins. Some useful test cells can be purchased especially for screening and particularly for the identification of antibodies. Others, such as A₁ and B, should be harvested locally, and may be from either Rh-positive or Rh-negative donors.

It is equally important to control all lots of antisera when placed in use. The need to control antiglobulin serum cannot be overemphasized. A good rule of thumb is to run both positive and negative controls once on each shift and again any time a new vial is put into use. In general, and with all reagents, proper storage temperature and refrigeration when not actively in use is a cardinal rule to be followed. Specificity, potency, and avidity are the parameters of control that will assure accuracy of results. A system of periodic testing for these parameters will assure continuous accuracy and control of the quality of results obtained on the bench.

The Antiglobulin (Coombs') Test

The antiglobulin test is an exceedingly useful tool by which we can detect the presence of incomplete or blocking antibodies (see page 9). The direct antiglobulin test detects in vivo coating or sensitization of

erythrocytes. The indirect antiglobulin test detects in vitro sensitization. Positive tests by either technique indicate nothing more than the presence of sensitized or "coated" cells. The direct antiglobulin test is helpful in the diagnosis of erythroblastosis, auto-immune hemolytic anemias, hemolytic transfusion reactions, and the study of drug or chemical intoxications involving hemolysis. The indirect antiglobulin test, by far the most frequently used, is an essential step in the routine crossmatch test, is helpful in screening donor and recipient serums for the presence of irregular antibodies, and in the identification of antibodies so found. This test is also frequently used when studying less common blood group systems in the pursuit of genetic, anthropologic, or forensic data.

Most of the pitfalls mentioned thus far for other kinds of serum also pertain to antiglobulin reagents. Thus, proper refrigeration, protection against contamination, and continuous quality control for proof of activity cannot be ignored. As has also been emphasized before, meticulous attention to the manufacturer's instructions for use is mandatory. While the NIH standards for its manufacture are protective to the consumer, they cannot guarantee success at the time of use.

False positive reactions may result from:

Wharton's jelly contamination of cord samples.

Bacterial contamination of antiglobulin serum, saline, test cells, or patient's cells (as in septicemia).

Colloidal silicate or other chemical contamination of saline.

Excessive centrifugation.

Resurgence of species-specific agglutinins removed during manufacture of antiglobulin serum.

Improperly cleaned glassware.

False negative reactions may result from:

Improper washing of test cells (carry-over of minute amounts of globulin from serum: Undue delay in completion of test may allow elution of coating antibody).

Failure to thoroughly mix the cells after each washing.

Failure to remove as much of the saline as possible after last washing before adding antiglobulin serum.

Failure to add antiglobulin serum to the test system.

Inactive antiglobulin serum (improper storage, contamination with serum-soiled droppers, etc.).

Loss or destruction of complement (serum specimens heat-inactivated or too old).

Another effect of intercurrent disease in a patient (such as septicemia causing false positive antiglobulin test) has been found to be

responsible for false negative antiglobulin tests. As a rule, three or four saline washes will remove the residual serum and avoid neutralization of the antiglobulin serum. In multiple myeloma, however, the marked increase in globulin associated with reversal of the A/G ratio may prove difficult to wash out. Amounts as small as 0.0001 ml of serum seem capable of neutralizing the amount of antiglobulin serum ordinarily used in a single test. Thus, there is valid reason for the laboratory staff to be advised of the presence in the hospital of patients with certain problems which may affect the validity of laboratory tests.

The use of commercial or in-house prepared "coated" or pre-sensitized cells as a check on antiglobulin tests regarded as negative has been proposed as an additional means of quality control (see recommendation for control of antiglobulin, page 20). The confirmation depends on the fact that there remains in a negative test unbound antiglobulin serum which agglutinates the coated cell. It certainly does indicate that the antiglobulin serum was added to the system and that it is reactive. In very weak positive indirect antiglobulin tests, however, which may incorrectly have been regarded as negative, the coated cell confirmation test will not detect the false negative result. More often than not, the coated cell will provide a substantial margin of confidence in the negative test, and its use can be recommended with the caution mentioned above.

Special Problems

Up to this point we have been concerned with the technical problems and pitfalls which are part of a "way of life" for the blood bank laboratory bench worker. A blood bank by today's concept is an installation capable of collection, processing and storage of human blood. But it is also more than this: A blood bank may ship its product to a remote geographic point, prepare it for infusion to a patient, process it into fractions, divert it at the end of a brief period of shelf life to a salvage program, or simply dump it down the drain. An installation which transfuses patients in addition to any of the other activities listed above is more precisely referred to as a transfusion service. Irrespective of the part or whole of the operation we may be concerned with, it is axiomatic that we strive to put the best possible product on the shelf, provide it with expert care during its period of usefulness, and get it where it ultimately may be required with unerring accuracy.

In order to insure the expertise and level of accuracy deemed necessary, comment on a few special problems may emphasize a

number of pitfalls that can be avoided. What has been said thus far has generally pertained to gaining information about units of blood coming into a bank. Most of it applied equally as well to gaining similar information about potential recipients of blood. We can, therefore, quite properly recommend an acceptable crossmatching procedure, the final test for compatibility before a recipient is transfused.

EXCEPT WHERE THE DELAY MAY RESULT IN LOSS OF LIFE, THE CROSSMATCH TEST MUST BE PERFORMED BEFORE A UNIT OF BLOOD IS TRANSFUSED. A major crossmatch (recipient serum and donor cells) is required. The minor crossmatch (donor serum and recipient cells) may be omitted if all donor units and recipient samples have been screened and are thus shown to be free of irregular antibodies.

Recommended Compatibility Test

The major crossmatch shall include tests in saline or serum and the indirect antiglobulin (Coombs') technique. The crossmatch must be capable of detecting both natural and immune antibodies. The indirect antiglobulin technique must include the use of NIH-approved antiglobulin serum in a manner recommended by the manufacturer to achieve these ends. It is optimal also to include albumin-serum or enzyme techniques in addition to the saline and indirect Coombs' techniques cited earlier. By and large, the average acceptable cross-matching procedure requires one hour.* (See note on page 24.)

Use of serum not heated-inactivated and not older than 36-48 hours is mandatory to insure the presence of sufficient complement to permit antibodies dependent upon this substance to react. Because of the anti-complementary effect of many anticoagulants, plasma should not be used.

Emergency Crossmatch Tests

When delay in transfusion would compromise survival, the following points are to be emphasized. When A-B-O and Rh type are unknown, the use of group O, Rh-negative blood without crossmatch is permissible. When this becomes necessary, the physician responsible for the case must indicate by signature the urgent nature of the case and acceptance of responsibility for this decision. As promptly as possible, the standard crossmatch is to be performed and the

results of this test conveyed to the attending physician even though transfusion has been started.

When time is sufficient that the A-B-O and Rh type can be determined, group-specific blood may be issued without crossmatch under conditions and provisions indicated above. It cannot be overemphasized that no substitute "short" crossmatch technique capable of protecting the recipient has yet been devised.

***Note:** Small and large, the average acceptable crossmatching procedure requires one hour. This includes receipt of patient's blood specimen, blood grouping and typing, preparation of crossmatch, incubation time, performance of antiglobulin test including sufficient washing of erythrocytes, completion of transfusion request forms, entry in blood bank ledgers and notification of ward personnel that the unit or units of compatible blood are ready for issue. Whereas, a period of 15-30 minutes allows for detection of a majority of antibodies that will produce destruction of erythrocytes in a patient, certain antibodies may require longer incubation periods. Examples of these are found in the Kidd and Duffy blood group systems. Our one hour procedure includes much more than the period of incubation and is recommended to prevent the issue of incompatible blood caused by short cuts of serologic technic, administrative procedure and other sources of clerical error.

Massive Transfusion

Patients requiring very large volumes of blood in relatively short intervals of time (15 to 30 units in 4 to 6 hours, for example) may be expected with some degree of frequency to demonstrate stubborn oozing once the acute loss has been controlled. Somewhat analogous to the defibrination associated with abruptio placentae in the obstetric patient, massive bleeding in trauma patients appears to be associated with extensive soft tissue damage. When laboratory tests reveal lowered fibrinogen levels, administration of this fraction is without doubt indicated. If fibrinogen is unavailable or its level not depressed, however, fresh frozen plasma (or if whole blood is indicated, units not more than a day old) may be expected to achieve hemostasis. A detailed discussion of component therapy, particularly as it affects the hereditary bleeder, is beyond the scope of this presentation.

In addition to bleeding associated with massive transfusion, an additional pitfall may be encountered if subsequent crossmatches are required for cases other than group O who have been given large volumes of group O blood. One now will be confronted with two different cell populations in the recipient sample, and although unlikely, there may be transient irregular antibody in the recipient serum. Use of group O packed red cells otherwise compatible with the recipient will introduce the least hazard with continuing need for transfusion. If the clinical situation demands whole blood, the packed red cells may be suspended in AB fresh frozen plasma screened for freedom from irregular antibodies. This maneuver will not only provide coagulation factors and restore volume, but it will also dilute the anti-A and anti-B antibody remaining with the plasma of the packed cells. To minimize the risk of contamination in processing donor blood for problems such as this, it is to be emphasized that multiple-pack, closed-system equipment must be used. It is also important to adhere to schedules of processing wherein transfusion immediately follows reconstitution of packed cell units of blood.

Multiple Transfusion

In contrast to the massively transfused recipient, the multiple-transfused patient may reach an equally large volume transfused, but his need extends over a matter of several days rather than a few hours.

It is important to perform the tests for compatibility on cross-match samples obtained fresh each day transfusion is given. This

will assure the presence of complement and provide the opportunity to recognize irregular antibodies that might result from previous transfusion.

A slightly different problem is presented by the patient with aplastic anemia or other long-term need for blood. These patients may begin to have severe febrile response to each unit transfused (perhaps as the result of leuko-agglutinins) and if transfused long enough, may develop classical irregular agglutinins. Reaction resulting from leuko-agglutinins can be minimized by the use of buffy-poor blood, prepared just prior to transfusion by centrifugation and removal of the white cell mass. Vigilance in the search for the earliest appearance of irregular isoagglutinins leads to the need to identify any such antibodies which appear. Selection of donors excluding these antigens then becomes an obligation in the selection of blood for subsequent transfusion.

Two consistent, though not frequent, pitfalls involve this group of massive- and/or multiple-transfused patients. Crossmatch samples from heparinized recipients (most often open-heart cases) have the peculiar propensity of forming continuously recurring gel in their serum. We can recommend no simple solution unless it is the judicious use of protamine or ionized calcium, bearing in mind that the resulting serum may be diluted or of altered electrolyte composition. The second group of patients are those on renal dialysis who are subjected to nephrectomy, transplantation, or other surgical procedures. These patients consistently ooze postoperatively until they are given immediately fresh (not more than one hour old) whole blood or fresh frozen plasma. A single unit of either product may have a dramatic effect.

Exchange Transfusion

The classic application of this procedure is in the treatment of erythroblastosis. Every effort must be made to crossmatch against maternal serum for this purpose because the offending antibody is in this serum and it will be of greater titer than in the infant's blood. When maternal serum cannot be obtained, the crossmatch may be performed on the baby's serum.

Additional applications of this procedure have been in drug or chemical intoxications where peritoneal lavage or renal dialysis may not be feasible, and more recently for management of hepatic coma.

Management of Suspected Hemolytic Transfusion Reactions

In spite of meticulous care and close attention to procedural detail, it appears inevitable that a small number of undesired reactions to transfusion may occur. Among several classes of transfusion reactions, those which produce hemolysis are the most dangerous, and this type may cause the death of a recipient. We have, for this reason, long accepted the responsibility for re-examination of tests on all patients in whom hemolytic transfusion reactions may be suspected. In our zeal to close all possible avenues of error, we have defined a time-consuming set of repetitive maneuvers and serologic tests that fail to lead quickly to answers that are necessary to care properly for these patients. There can be but little quarrel that the data required by the Minimum Standards are useful or that they should be derived. It would appear, however, that the first order of business is to provide a yes or no answer as quickly as possible to the simple question, "has a hemolytic transfusion reaction occurred?" An affirmative answer to our question means that the patient must be placed under treatment at once. The rechecks recommended by the Minimum Standards should then be completed as promptly as possible, and may now be carried out without further comprising the recipient's chances of surviving a hemolytic reaction to transfusion.

The existence of incompatible blood transfusion disease (IBTD) can be established quickly and beyond doubt by demonstrating a positive direct antiglobulin (Coombs') test and/or the presence of free hemoglobin in the serum of the recipient. In a recent reappraisal of the diagnosis and treatment of IBTD, Nalbandian, Mader, and Margolis emphasize the need for a fresh approach on the part of clinicians and blood bank workers alike. In the interests of saving the lives of recipients receiving incompatible blood, these authors insist on the need for speed, simplicity, and certainty. They contend, and we agree, that the chances of survival vary inversely with time between the infusion of incompatible blood and the start of treatment. Treatment with mannitol, closely in accord with the recommendations of Barry and Crosby and of Parry has been endorsed. Nalbandian and associates emphasize the need for speed and propose that the simple visual inspection of post-transfusion serum is adequate to confirm significant hemoglobinemia. A direct antiglobulin test can be performed in very few minutes on the same specimen of blood. They have thus fulfilled their criteria of speed and simplicity. Concerning certainty: The diagnosis can be established or excluded with equal accuracy. Instructions for the diagnosis and treatment of incompatible blood transfusion disease have been placed by Nalbandian and

his associates at each nursing station throughout the hospital where transfusion may be given.

In our estimate, the salient areas to the avoidance of pitfalls concern: 1) communication between personnel in the patient care area and the laboratory. This must be established from the moment a reaction is first suspected, and maintained throughout the interval of observation and/or treatment; 2) communication within the laboratory between bench workers and physician supervisor, irrespective of the hour or the day of the week a reaction is recognized; 3) care with technique of obtaining samples required for the study, and 4) documentation of samples including relationships in time to infusion of the offending transfusion and to each other.

As we have indicated above, once the diagnosis is established, treatment with mannitol must be prompt and directed toward maintaining renal flow approximately 100 ml/hour. With respect to technique, the single most significant pitfall to be avoided is the artificial induction of hemolysis in the first post-transfusion specimen of blood. This can be prevented only through careful venipuncture following minimal stasis and probing, and by the use of chemically clean and dry equipment.

The clear designation of time and date, along with proper patient and source of specimen identification, is the only means by which accurate reconstruction of events can be achieved. Properly organized, the blood bank laboratory will have saved all crossmatch specimens and pilot tubes for a minimum of 7 days following transfusion. These specimens, along with samples from the container involved in the reaction and post-transfusion samples, now can be rechecked for group, Rh type, and compatibility testing. When the evidence suggests that a hemolytic reaction has in fact occurred, antibody titrations before and after transfusion, especially the pertinent natural, regular isoantibodies, will provide confirmatory data. Culture of pertinent blood specimens is also to be performed. Quantitative haptoglobin estimates under certain conditions provide another parameter of evaluation.

When other accidents appear to be responsible for a hemolytic transfusion reaction, e. g., gross bacterial contamination or massive hemolysis of a donor unit, whatever the cause, the sequences of communication and notification of top-level professional personnel are even more important because these patients may die in a very short time. For the latter problems, the nature of specimens and the way in which they are to be handled may, of course, be modified to fit the circumstances. The necessity for accurate documentation and labelling of materials to be studied remains the same.

Since the occurrence of transfusion reactions is contrary to the result we have set out to achieve, each such instance must serve as a teaching and reorientation exercise for all personnel in the department. It is only by this means that every member of the staff can rededicate himself to minimizing the risks of blood transfusion.

Glossary of Terms Used in Immunohematology

Absorption:	Removal of antibodies from a serum by red cells bearing the appropriate antigen receptors. Sera so treated are said to be absorbed.
Adsorption:	This term has been used synonymously with <u>absorption</u> in blood grouping literature. Conventionally it has been applied both to gas-liquid systems and liquid systems containing particulate solids.
Agglutinates:	Clumps of adhering red blood cells.
Agglutination:	The clumping of red blood cells resulting from an antibody (agglutinin) specific for an antigen on those cells.
Agglutinin:	An antibody capable of agglutinating red blood cells containing the corresponding specific antigen (agglutininogen).
Agglutininogen:	An antigen on red blood cells which is capable of inducing an agglutinating antibody in a suitable host.
Allele, Allelomorph:	One of two or more genes which determine alternative characteristics in inheritance, located at the same relative loci on a pair of homologous chromosomes.
Amorph:	A gene without determinable effect.
Antibody:	A substance appearing in the serum of a host in response to the introduction of an antigen. The resulting antibody reacts specifically with the antigen introduced.

Anticoagulant:	A substance which prevents clotting when added to whole blood.
Antigen:	Any substance which, upon being introduced into an individual lacking that substance, is capable of inducing the production of an antibody in that person.
Antigenicity:	The relative capacity of several antigens to induce immune response. When comparing two or more antigens, that inducing the most intense antibody response is said to be the most antigenic.
Antiglobulin:	An antibody obtained from animals (often rabbits or goats) immunized with whole human serum or human globulins. Such serum in the antiglobulin test is capable of detecting cells coated with incomplete human antibody by producing agglutination.
Autoagglutinin:	An antibody in the serum of an individual that reacts with his own red cells; it may also agglutinate the red cells of most other persons as well.
Autosome:	Any chromosome except a sex chromosome.
Avidity:	Ability of an antibody to agglutinate red cells rapidly and completely. Under controlled conditions of cell concentration, reaction temperature, and size of resulting agglutinates, avidity is the determination of the number of seconds for reaction to begin and to become complete.
Back-Type or Confirmation Test:	Abandoned terms - see <u>Serum Grouping</u> .
Bernstein's Theory:	Provided mathematic proof that the inheritance of the A, B, and O antigens was controlled by three allelomorphic genes in accordance with Mendelian law. The fourth and fifth alleles A ₂

and A₃ (both are subgroups of A) were later also found to fit the theory.

Blocking Antibody:	An incomplete antibody capable of coating red cells possessing its specific antigen, but incapable of causing agglutination of these cells when suspended in saline. Once heavily coated, these cells would not be agglutinated by a complete antibody of the same specificity because they have been "blocked."
Blood Group, Sex-Linked:	A sex-linked blood group antigen (Xg ^a) has a frequency of 62% in males and 89% in females. Phenotype symbol: Xg(a ⁺).
Blood Factor:	A serological specificity of an agglutinin.
Blood Grouping:	The classification of blood specimens into groups (or types) on the basis of the blood factors or agglutinogens which they contain.
Blood Group Substances:	Substances of human or animal origin that are closely related, if not chemically identical, to red cell antigenic material and have the ability to neutralize specific antibodies stimulated by the red cell antigen.
Blood Group System:	A system of related blood group factors or agglutinogens such as the A-B-O system, the M-N-S system, and the Rh-Hr system.
Bombay Bloods:	The Bombay, or O _h bloods, are identified by reactions similar to group O. The serum contains anti-A, anti-B, and anti-H. The red cells are not agglutinated by anti-A, anti-B, anti-A,B (group O), or anti-H.
Bovine Albumin:	Fraction V of the Cohn procedure for separation of bovine plasma.
Chimera:	A rare condition in which an individual has two genetically different blood groups, e. g., group O and group A. This phenomenon was

recognized in cattle long before it was first found in man.

- Chromosome:** One of the small bodies, ordinarily definite in number, in the nuclei of cells of a given species, into which the chromatin resolves itself during mitosis. Distinguishable only at the time of cell division, it is made up of a series of genes in linear arrangement.
- Coated Cells:** Incomplete antibody may attach to the surface of a cell bearing antigen for that antibody without producing agglutination; such cells are said to be coated.
- Codominant Genes:** Genes comprising a heterozygote which are capable of expressing themselves. Blood group AB, in which A and B can be detected in the presence of each other, are examples of codominant genes.
- Cold Agglutinin:** An agglutinin showing optimum reaction at 4°C, with progressively less reactivity as the temperature is increased. While many react at 20°C, few do so at 37°C.
- Complement:** Factors present in fresh serum necessary for hemolysis of red cells sensitized by hemolysins. The complement activity is destroyed by heating at 56°C for 30 minutes.
- Complete Antibody:** An antibody which causes the agglutination of saline-suspended red cells possessing the specific antigen. It may also agglutinate red cells suspended in other media.
- Conglutinin:** (Wiener) A colloidal aggregate of serum proteins which, when adsorbed by cells which have been sensitized (or coated) by their specific univalent antibodies, causes them to stick together (conglutination).

Coombs' Test:	A test using an antiglobulin serum to detect globulins coating the surface of red cells.
Cord Blood:	Blood obtained from the umbilical cord at birth. All cord blood is of fetal origin.
Crossing Over:	Exchange of genetic material between chromosomes at chiasmata.
Crossmatch:	The <u>in vitro</u> pre-transfusion tests for compatibility between a patient and a prospective donor.
Direct Coombs' Test:	An antiglobulin test used to detect the presence of antibody coated on red cells <u>in vivo</u> such as hemolytic disease of the newborn and acquired hemolytic anemia.
Dizygotic or Fraternal Twins:	Twins arising from two separately fertilized ova.
Dominant Gene:	When two different alleles (genes) are present, one may completely suppress the action of the other and is said to be dominant. The other allele (gene) is called recessive. It should be noted that genes are not always completely dominant or completely recessive.
Eel Serum:	A source of anti-H serum.
Eluate, Elution:	The product or process of removing antibody from red blood cells. Elution is usually performed on cells suspended in saline by heating at 56°C for 10 minutes. Media other than saline may also be used. Antibody so released from coated or agglutinated red cells can be detected in the supernate (which is the eluate).
Enzyme Techniques:	Techniques employing proteolytic enzymes that partially digest the surface of erythrocytes. Such treatment often enhances agglutination with weak or incomplete antibodies. In some systems enzymes may destroy reactivity.

Epistasis:	Enhanced expression of a gene by the presence of another gene on a different chromosome.
Family Antigens:	Red cell antigens which are confined to the members of one family.
Ficin:	Proteolytic enzyme obtained from figs.
Fisher Nomenclature:	The rhesus antigens are described by the symbols C, c, D, d, and E, e.
Frozen Cells:	Preservation of red cells by use of glycerol-citrate and freezing or storing in liquid nitrogen.
Gene:	The unit of inherited material.
Genotype:	The genetic constitution of an individual for a particular blood group system. The sum of all genetic material inherited by an individual.
H Antigen:	Substance found in saliva of secretors which will neutralize anti-H agglutinins. A rough reciprocal relationship exists between A and H red cell antigens. For example, the more H the less A, the more A the less H. Excluding Bombay bloods, all O red cells are H-positive, most A ₁ cells are H-negative.
Hapten:	A substance having the serological properties of antigens in <u>in vitro</u> tests, but without antigenic action <u>in vivo</u> .
Heat-Stable Antibody:	An antibody which can withstand heating at 70°C for 5 minutes.
Hemagglutinins:	Antibodies which agglutinate red cells.
Hemolysin:	A substance causing rupture of red cells.
Hemolysis:	The process in which the red cell pigment (hemoglobin) is released into the surrounding

	medium following damage of the red cell membrane.
Hemolytic Disease of the Newborn (HDN):	Anemia of the newborn infant resulting from immunization of the mother to red cell antigens of her fetus. These immune antibodies formed by the mother cross the placenta and destroy the fetal red cells carrying the specific antigen.
Heteroagglutination:	The agglutination of red cells of one species of animal by antibodies obtained from another species.
Heteroagglutinin:	An agglutinin of one species of animal capable of agglutinating the red cells from another species.
Heterozygous:	Having two different allelic genes on the two corresponding loci of a pair of chromosomes. If different alleles are present on the two chromosomes, the cell is said to be heterozygous for that gene.
Homologous Chromosomes:	Two chromosomes which have identical loci in the same linear order.
Homozygous:	Having identical allelic genes on the two corresponding loci of a pair of chromosomes. If two genes on the homologous chromosomes are the same, the cell (organism) is said to be homozygous for that particular allele.
Hübener-Thomsen Phenomenon:	Panagglutination of red cells caused by contaminated serum. This is also known as bacteriogenic panagglutination.
Hyperimmune Antibody:	An antibody produced by repeated courses of immunization.
Hypertonic Saline:	Sodium chloride solution containing more than 0.85% sodium chloride. Red cells exposed to

	hypertonic solutions of NaCl lose water and shrink. They are then called crenated cells.
Immune Antibody:	An antibody produced by immunization.
Immunization:	The appearance of antibodies following the introduction of foreign antigens through blood transfusion, pregnancy, or parenteral injection.
Inactivation of Serum:	Destruction of the thermolabile constituents of complement by heating at 56°C for 30 minutes.
Incomplete Antibody:	An antibody which "coats" but does not visibly react with red cells containing the corresponding antigen is said to be incomplete. Other terms for such antibodies are: blocking antibodies, glutinoids, glutinins, and albumin antibodies.
Incubate:	To maintain at a specified temperature.
Indirect Coombs' Test:	A means of detecting the presence of an incomplete or blocking antibody in a specimen of serum.
Inhibition:	Prevention of the reaction of an antibody by addition of the antigen in a soluble form.
Isoagglutinin:	An antibody which reacts with red cells of the same species.
Isoimmunization or Isosensitization:	Immunization of an animal by antigens from the same species of animal.
Isotonic Solutions:	Solutions which have the same osmotic pressure or salt concentration as blood.
Landsteiner Law:	In adults, the serum contains those and only those agglutinins to the A and B antigens which are lacking in the red cells.

Linked Genes:	When two genes occur on the same chromosome they are said to be linked; they are, therefore, inherited together, unless separated by crossing over.
Locus:	An area on a chromosome denoting the location of a gene or one of its alleles. The position a gene occupies on a chromosome.
Lyophilized Serum:	A serum which has been dried under vacuum while frozen.
Major Crossmatch: (Compatibility Test)	Mixture of patient's serum and donor's red blood cells.
Minor Crossmatch:	Mixture of patient's red blood cells and donor's serum.
Multiple Allelomorphs:	A series of more than two genes, all alternates.
Natural Occurring Agglutinin:	An agglutinin present in a serum which has occurred without any obvious antigenic stimulus. It may be, however, that so-called natural occurring antibodies are in fact hetero-immune in origin.
Neutral Serum:	AB serum free of detectable isoagglutinins of any kind.
Nonsecretor:	A person whose saliva contains neither A, B, nor H substance and whose red cells are usually Le(a+).
Nonspecific Agglutinin:	An agglutinin without a specific antigen active at low temperature which disappears on warming. It can be adsorbed out at 4°C.
Nonspecific Reaction:	A reaction between red cells and serum not due to a specific antigen-antibody system.
N. T. :	An abbreviation used to indicate "not tested."

O Antigen:	The theoretical product of the Bernstein O gene in the A-B-O blood groups.
Ox Serum:	Serum from bovine blood.
Packed Red Cells:	The concentrated suspension of red cells obtained from whole citrated blood after sedimentation of the cells either by gravity or centrifugation. A unit of red cells following removal of the plasma.
Panagglutinable Cells:	Red cells that are agglutinated by all adult sera at 4°C and sometimes at 20°C irrespective of the A-B-O groups.
Panagglutinin:	An agglutinin which causes agglutination of all red cells regardless of blood group.
Panel of Cells:	A set of separate samples of red cells selected for their antigenic makeup used to identify antibodies.
Papain:	A proteolytic enzyme obtained from the papaya fruit.
Paternal Blood Group:	Blood group of the father.
Paternity Tests:	Blood group studies on mother, child, and alleged father.
Pedigree:	Graphic representation depicting the relationship of members and the inheritance of a genetic marker (blood group) through several generations of a family.
Phenylthiocarbamide:	An organic chemical used to distinguish the inherited ability to taste or not to taste this substance.
Phytohemagglutinins:	A mucoprotein extracted from certain seeds which agglutinate red blood cells.

Plant Lectins:	Class of hemagglutinins obtained from seeds.
Plasmapheresis:	A procedure in which whole blood is removed from a donor, the plasma extracted following centrifugation, and the residual-packed red cells are returned to the donor by auto-transfusion.
Pooled Cells:	A mixture of two or more specimens of blood.
Polyvinylpyrrolidone (PVP):	A synthetic macromolecular plasma volume expander which is also useful for suspending test cells for the detection of incomplete antibodies.
Precipitin Test:	A specific serological test on antigens in solution in which an antigen-antibody reaction results in the appearance of a finely divided visible precipitate.
Propositus:	That member of a family subjected to genetic study whose test results first attracted the interest of the investigator.
Proteolytic Enzyme:	A class of substances capable of digesting protein and used in blood group serology for digestion of red cell surfaces.
Protocol:	The original record of the results of scientific tests.
Prozone Reaction:	Failure of a serum to produce agglutination in its more concentrated form which, upon being further diluted, gains this capacity. It is thought to be the result of antibody excess or the presence of blocking antibodies.
Pyrogen:	Thermostable, filterable substances, possibly of bacterial origin. Their presence in intravenous solutions causes febrile reactions following injection.

Rh_{null} Bloods:	Very rare persons whose red cells contain no detectable antigens in the Rh-Hr system. This is of two types: one type is due to suppressor genes and the other is due to a special allelic gene \bar{r} , i. e., such type rh persons are of genotype $\bar{r} \bar{r}$.
$\bar{R}h_0$ Bloods:	Blood from individuals lacking the contrasting blood factors $rh' - hr'$ and $rh'' - hr''$.
Rouleaux Formation or Pseudo-agglutination:	A property of serum (not an antibody) which causes red cells to stack like a pile of coins, thus superficially resembling agglutination.
Saline Agglutinin:	A "complete" antibody, or one capable of producing agglutination of cells suspended in saline.
Sensitized Red Cells:	Red cells coated with antibody globulin.
Sequestrene (EDTA):	Disodium dihydrogen ethylenediaminetetraacetate dihydrate, an anticoagulant.
Serum Grouping:	The determination of antibodies in a serum which can establish the A-B-O group of that person, or verify the results of grouping tests performed on the cells of the same blood specimen.
Sibs or Siblings:	Progeny of the same parents: brothers or sisters.
Specificity:	The term applied to the reaction of an anti-serum and its antigen on erythrocytes.
Subgroups:	A term normally confined to the alleles of A such as A_1 , A_2 , A_3 , A_4 , A_x , etc.
Survival Studies:	The measurement of the life span of transfused red cells.

Thermal Amplitude:	The highest temperature at which a serum will react with the appropriate red cells.
Titer:	The greatest dilution of a serum which still has sufficient antibody present to react with the corresponding antigen. It is expressed as the dilution 1:2, 1:4 or more commonly as the reciprocal of this dilution 2, 4, etc.
Trypsin:	A proteolytic enzyme recovered from pancreatic juice. It is useful for the detection of incomplete antibodies, and may assist by enhancing many weakly reacting antibodies.
Warm Agglutinins:	Antibodies with a thermal optimum of 37°C.
Washed Cells:	Cells freed of plasma or serum by repeated centrifuging through fresh volumes of normal saline.
Water-Soluble Blood Group Substances:	A, B, or H substances found in the body fluids or secretions.
Zygoty:	The estimation of whether an individual has inherited like (homozygous) or unlike (heterozygous) pairs of genes for a particular trait.

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Personal Notes

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A N N E X T
SCIENTIFIC EXHIBITS

SCIENTIFIC EXHIBITS

Presented at Annual Meetings of
AMERICAN ASSOCIATION OF BLOOD BANKS

1964

A Simple Manifold Washing Process for Preparing Erythrocytes for the Antihuman Globulin Test (Figures 1 and 2)

MARY B. GIBBS, MAJOR FRANK R. CAMP, JR., Walter Reed Army Institute of Research, Washington, D. C.

A new manifold erythrocyte washing process was demonstrated. The entire washing process is carried out without removing test tubes from the centrifuge. Advantages of the method are a more rapid and effective wash and standardization of technic.

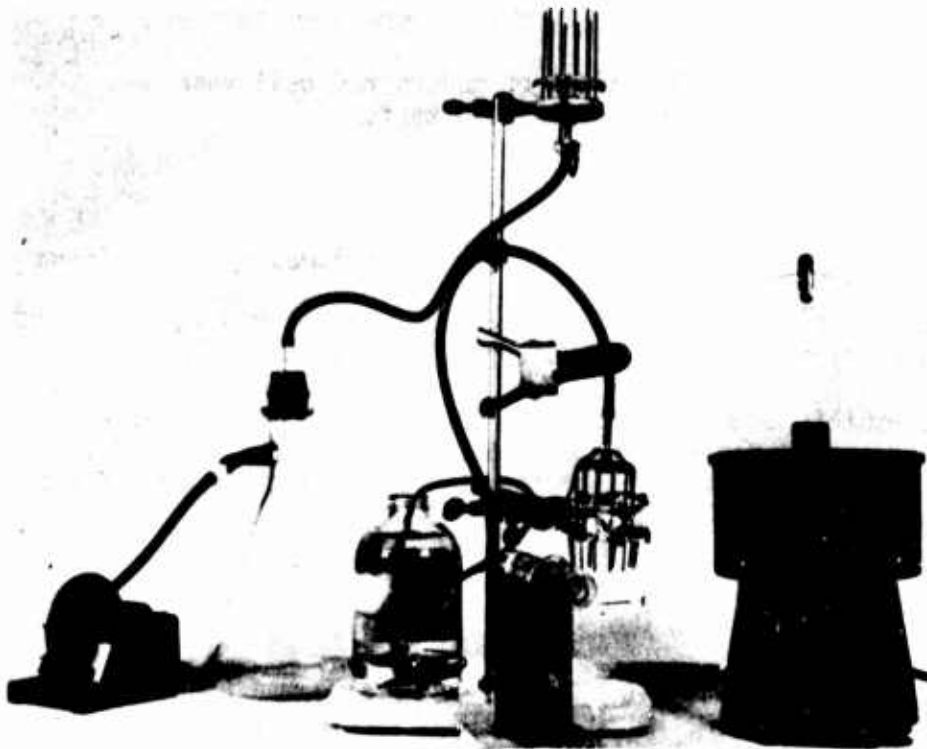


Fig. 1. Prototype of modern red cell washers for the anti-globulin test.

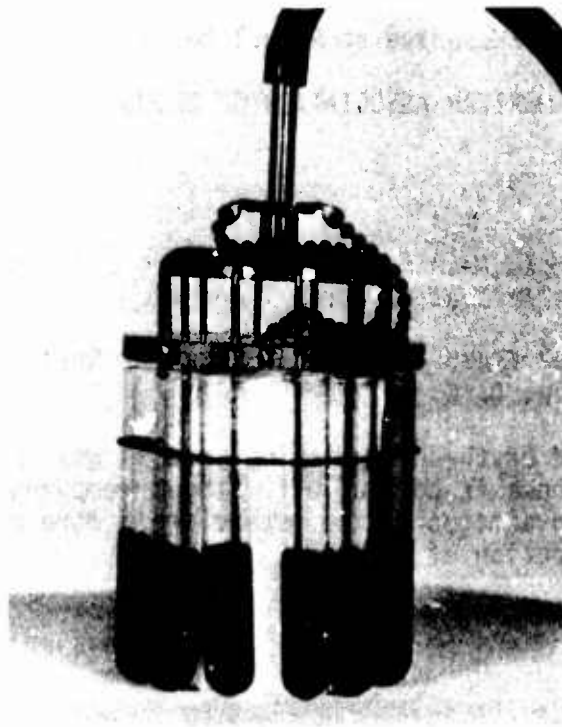


Fig. 2. Prototype of modern red cell washers for the antiglobulin test.

1965

Red Cell Osmotic Fragility Recording Produced by a Densitometer

CPT TURMAN E. ALLEN, JR., MAJ CHARLES E. SHIELDS, MAJ FRANK R. CAMP, JR.,
Walter Reed Army Institute of Research, Washington, D. C.

The exhibit demonstrated a machine adapted from standard equipment, and illustrated a rapid, simple method of depicting red cell fragility. The exhibit showed actual recordings of normal and abnormal fresh blood and of stored blood.

1966

Translation of Rare Manuscripts (Figure 3)

MAJ CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., MAJ GEORGE IKEDA, CPT
DAVID G. COURTENAY, CPT MAX H. MC LAIN, US Army Medical Research Laboratory,
Fort Knox, Kentucky.

The Transfusion Research Division featured the translation of rare manuscripts in the field of blood grouping, blood banking, and blood

transfusion which was undertaken to provide present-day research institutions with the original work of the "old masters." The work of Dr. Karl Landsteiner was investigated in detail to bring the full impact of his genius to all in the various aspects of immunology, immunohematology, and immunochemistry.

Military Blood Banking (Figure 3)

MAJ CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., MAJ GEORGE IKEDA, CPT DAVID G. COURTENAY, CPT MAX H. MC LAIN, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Illustrated, with words and color photography, was the updating of military blood banking in the pertinent areas of blood cell preservation, blood group automation, and training of military personnel. The ideas demonstrated were implemented from current research into practical blood banking operations and training programs.

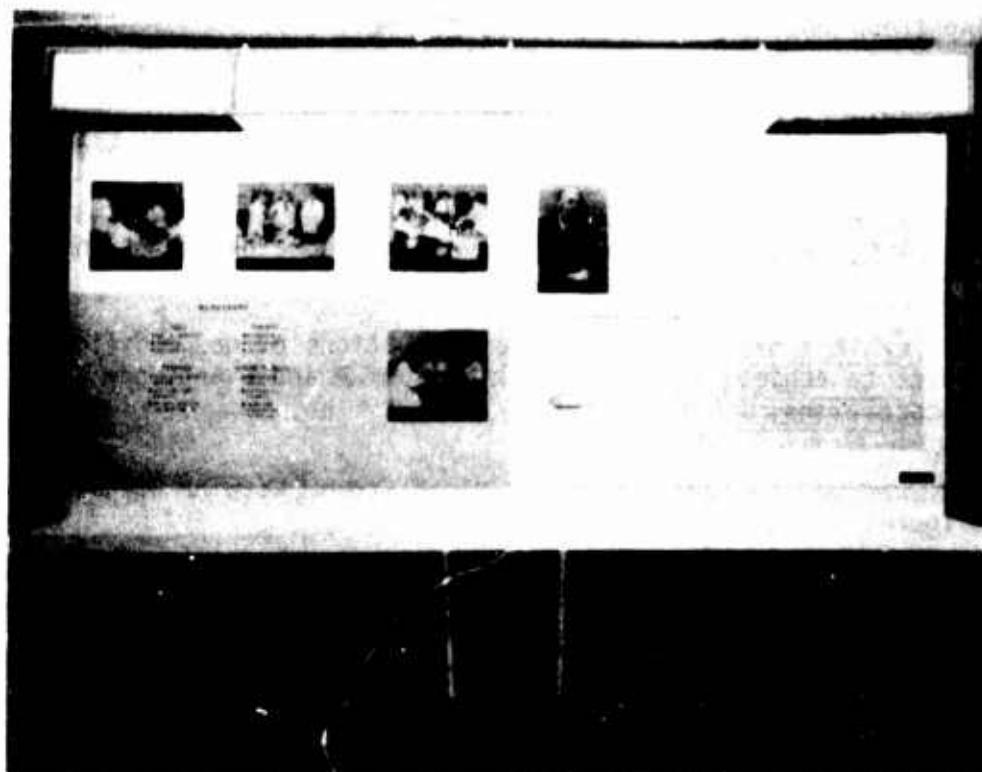


Fig. 3. Left: Training - military blood banking;
Right: Translation of rare manuscripts.

Universal Donor--A Military Problem?

MAJ CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., MAJ GEORGE IKEDA, CPT DAVID G. COURTENAY, CPT MAX H. MC LAIN, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Eliminating effects on the group O donor population was depicted on an electrical panel reflecting the imposed conditions of Rh, titer of isoagglutinins, hemolysins, and immune antibodies. *In vivo* results were presented to answer feasibility problems.

Plasma Separation in the Military

MAJ CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., MAJ GEORGE IKEDA, CPT DAVID G. COURTENAY, CAPT MAX H. MC LAIN, US Army Medical Research Laboratory, Fort Knox, Kentucky.

A plasma salvage pilot model, designed at Fort Knox and implemented in Europe for the US Army to provide fresh frozen plasma, plasma, platelet concentrates, and packed red cells, was displayed. Cryoprecipitate fibrinogen from single donor and plasmapheresis operations was an integral part of the model. Both *in situ* and mobile operations were described. The yields of commercial processing were shown in chart form, relating fresh and aged plasma yields, respectively.

1967

Titer Standardization for Antibody Screening (Figures 4 and 5)

CPT LOUIS J. REED, CPT LEONARD G. DAUBER, LTC CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., US Army Medical Research Laboratory, Fort Knox, Kentucky.

This exhibit provided working demonstrations of new modifications of technics to achieve reproducible titers of A and B antibody under various conditions.

RBC Survival Measurement of ACD Blood Stored With or Without Adenine (Figures 4 and 5)

LTC FRANK R. CAMP, JR., LTC CHARLES E. SHIELDS, CPT LOUIS J. REED, CPT LEONARD G. DAUBER, CPT HOWARD F. BUNN, CPT STEPHEN D. LITWIN, US Army Medical Research Laboratory, Fort Knox, Kentucky.

RBC survivals were measured between recipients receiving blood stored with or without adenine. In some storage periods, the same unit was divided to compare auto versus non-auto transfusions, with the latter subdivided into type specific and universal donor transfusions.

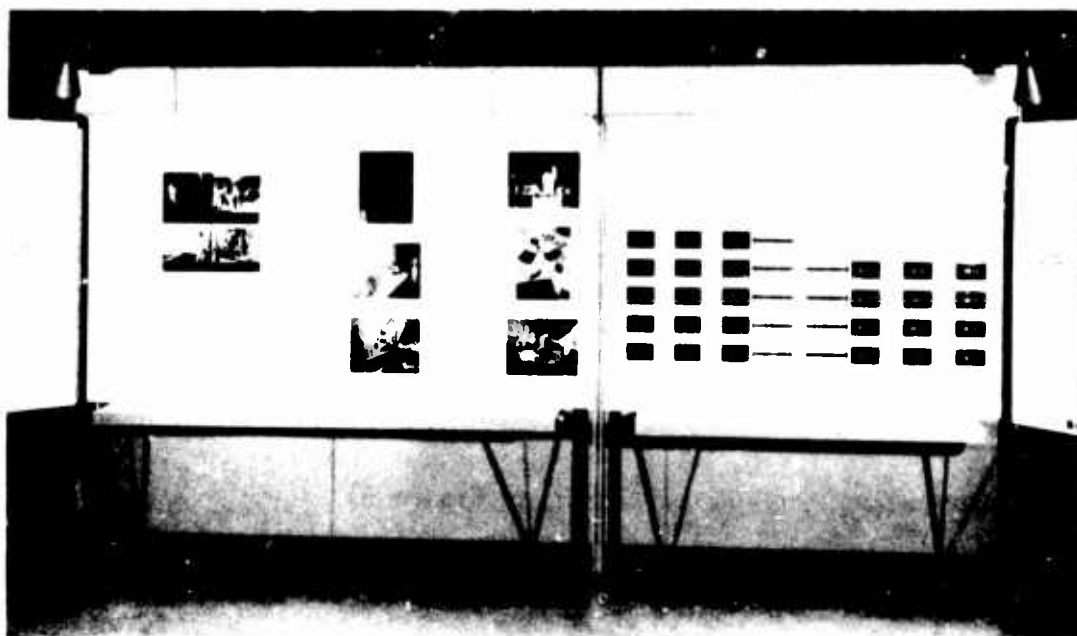


Fig. 4. Left: Titer standardization for antibody screening; Right: RBC survival measurement of ACD blood stored with or without adenine.

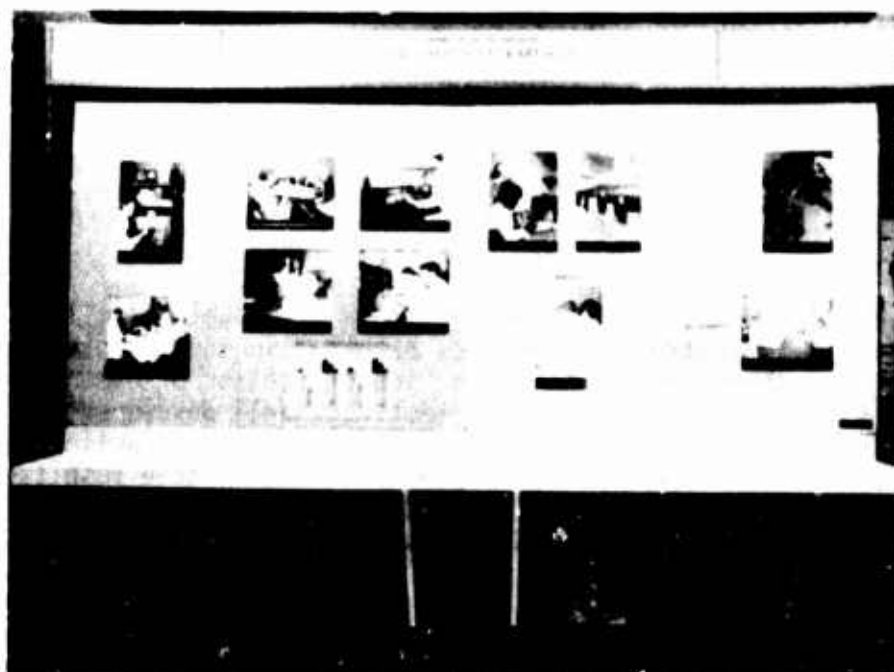


Fig. 5. Left: RBC survival measurement of ACD blood stored with or without adenine; Right: Titer standardization for antibody screening.

The Technical Aspects of Military Blood Banking in Technology

LTC FRANK R. CAMP, JR., LTC CHARLES E. SHIELDS, RICHARD A. WHEELER, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Areas under development in blood bank technology, equipment, and reagents were shown being incorporated into the total effort of the military blood program in providing the ring of safety--which is designed to provide the maximum protection for the donor, the blood, and the recipient.

1968

Prevention of Transfusion Reaction (Figure 6)

LTC CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., CPT DAVID K. HYSELL, COL RALPH H. FORRESTER, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Preventable reactions to transfusion usually involve considering three major areas of error: clinical, methodology, or reagents. Some examples of errors and possible means to reduce these were shown at this exhibit. Maintenance of adequate quality of Army control sera has been assigned to the Blood Transfusion Division and also is necessary in providing reliable forensic laboratory support. Included in this quality control are pyrogen testing, reagent comparison, and method evaluation.

Evolution of a Military Forensic Testing Laboratory (Figure 6)

MG JOSEPH M. BLUMBERG, LTC FRANK R. CAMP, JR., LTC CHARLES E. SHIELDS, CPT STEPHEN D. LITWIN, FRANK R. ELLIS, M.D., COL RALPH H. FORRESTER, US Army Medical Research Laboratory, Fort Knox, Kentucky.

The development of new technics and new approaches, facilitated by better scientific instrumentation, has resulted in the creation of a modern Forensic Testing Laboratory for identification of human remains, paternity studies, and medical-legal testing. Well developed technics, such as blood group serology and precipitin analysis, as well as newer approaches, like Gm typing of serum immunoglobulins, have resulted in wider applicability and reliability in the study of forensic problems. These technics were explained and discussed at this exhibit.

The Karl Landsteiner Centennial (1868-1968--A Compendium of Immunohematology Literature) (Figure 6)

MG JOSEPH M. BLUMBERG, LTC FRANK R. CAMP, JR., LTC CHARLES E. SHIELDS, FRANK R. ELLIS, M.D., COL RALPH H. FORRESTER, RICHARD A. WHEELER, US Army Medical Research Laboratory, Fort Knox, Kentucky.

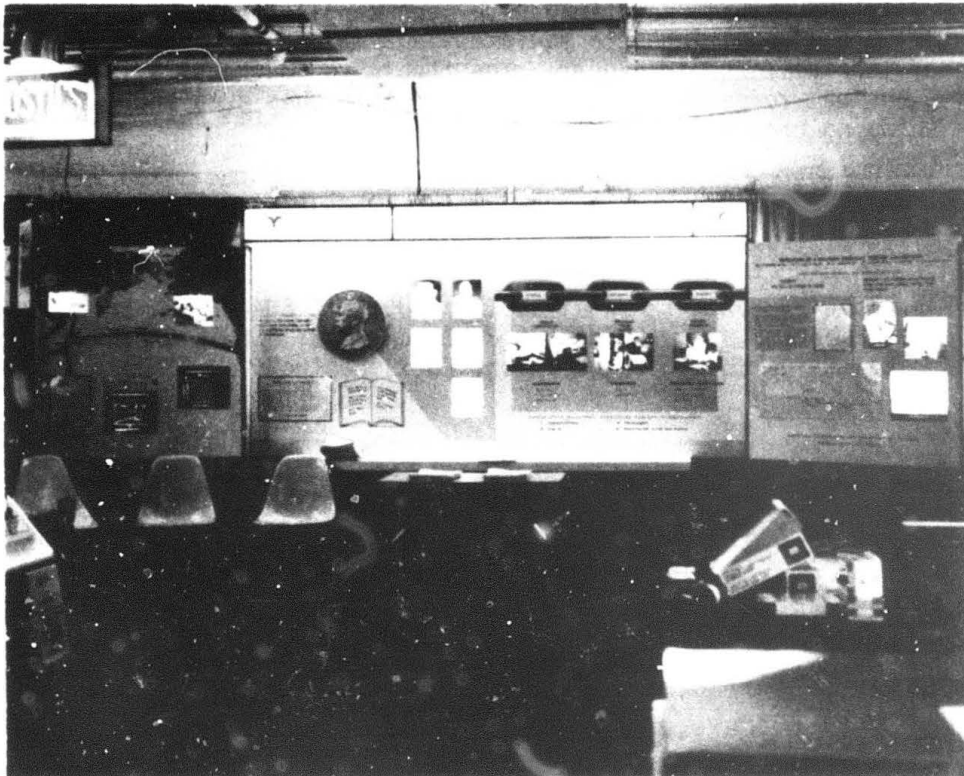


Fig. 6. Left: Overseas transport of blood; Center left: The Karl Landsteiner Centennial; Center right: Prevention of transfusion reaction; Right: Evolution of a military forensic testing laboratory.

The completion of four years of research to make available the largest single series of blood group immunology translations was described in this exhibit. A distinct feature of the presentation concerned the five students of Landsteiner and, equally important, the outstanding contributions recorded in the 20th Century scientific literature, attributed to Karl Landsteiner, the Father of Immunohematology. The ABO International Nomenclature of Landsteiner has been mandatory in the US Army since 1941. It is not generally appreciated that the military was a pioneer in this important standardization of the ABO nomenclature. At this exhibit, Landsteiner's original experiment was illustrated also.

Overseas Transport of Blood (Figure 6)

LTC CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR., DAILEY W. MC PEAK, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Military overseas operations have been supplied by blood collected from many areas requiring both equipment and methods to reduce the effects of long distance transport. The supply to Southeast Asia involved meeting unusual temperature ranges; research in this area and involving other shipping problems was illustrated.

1969

Patterns of Instruction in Blood Group Genetics (Figure 7)

LTC FRANK R. CAMP, JR., FRANK R. ELLIS, M.D., LTC CHARLES E. SHIELDS, MAJ TURMAN E. ALLEN, JR., RICHARD A. WHEELER, MARY JESSIE CRAYCROFT, US Army Medical Research Laboratory, Fort Knox, Kentucky.

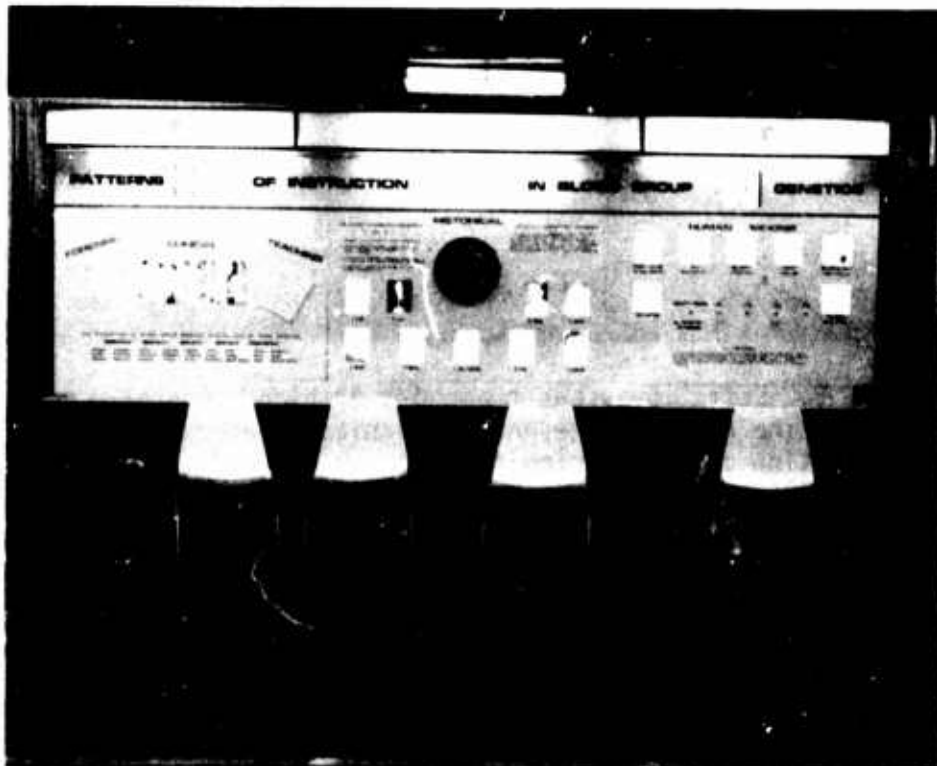


Fig. 7. Patterns of instruction in blood group genetics.

The role of basic genetics in creating a solid foundation in immunohematology and its various counterparts is undisputed. A complete monograph on blood group genetics, which treats all aspects of the subject, specifically designed for the student, did not exist. In an attempt to fill this void, a special training program was implemented at the Blood Transfusion Division, US Army Medical Research Laboratory, Fort Knox,

Kentucky, for the 52-week Blood Bank Fellowship Program. The scope and methods of instruction were presented in the exhibit which included proper emphasis on cytology, genetics, cytogenetics, statistics, embryology, anatomy, immunology, immunochemistry, immunoanthropology, and other related sciences. The implications of organ transplant are equally stressed, as well as other interesting and related developments, which make the subject of blood group genetics one of the most exciting and inspiring areas of scientific presentation.

New Aids for the Blood Bank Technologists (Figure 8)

MAJ TURMAN E. ALLEN, JR., LTC CHARLES E. SHIELDS, LTC FRANK R. CAMP, JR.,
US Army Medical Research Laboratory, Fort Knox, Kentucky.

A working exhibit demonstrated the use of two newly developed pieces of equipment for the blood bank. One is a manually operated electrical translating device to facilitate the reading of blood types on the filter paper strip of the automatic blood typing equipment. The other is a small hand-held microviewer for the observation of hemagglutination at 80 X magnification directly in the test tube.

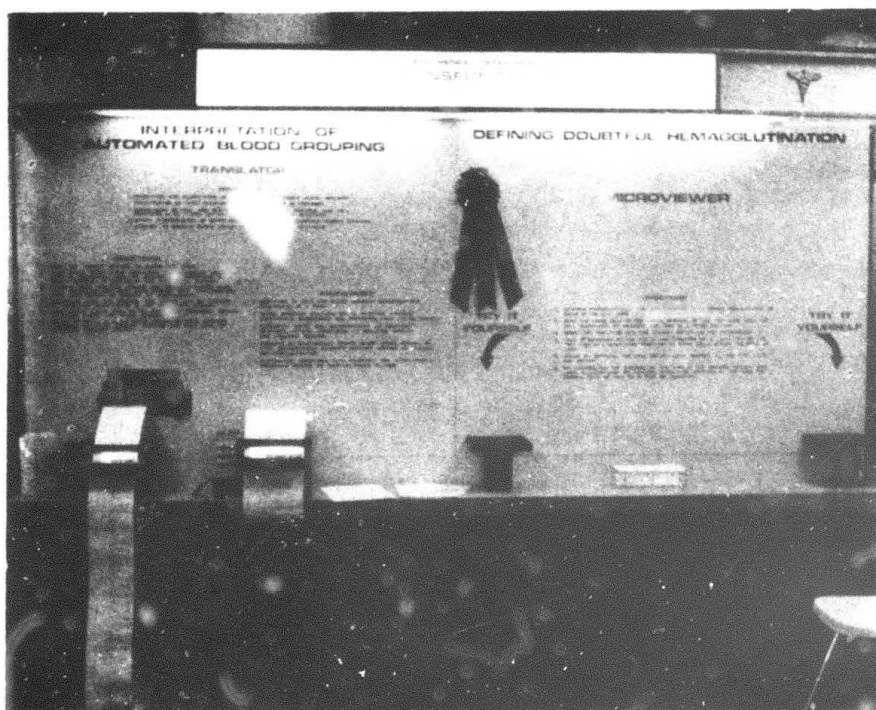


Fig. 8. New aids for the blood bank technologists.

Blood Cooling Logistics--Prototype Systems (Figure 9)

DAILEY W. MC PEAK, ARTHUR H. SCHIPUL, JR., GORDON F. MILLER, JOHN W. BURNS, LTC CHARLES E. SHIELDS, US Army Medical Research Laboratory, Fort Knox, Kentucky.

A blood collecting device which also cools the blood had been on trial. Fresh blood is sufficiently cooled at temperatures below 10 C for 10-14 minutes so that it can be shipped. The blood shipping box temperature, 4-10 C, can be affected by external temperature, especially radiant heat.

Quality Control Systems Applications in Blood Collecting Operations (Figure 9)

LTC CHARLES E. SHIELDS, MAJ TURMAN E. ALLEN, JR., LTC FRANK R. CAMP, JR., US Army Medical Research Laboratory, Fort Knox, Kentucky.

High quality unit collection in relation to donor reactions can be analyzed using both quality control charts and management systems. The exhibit demonstrated application of these in one blood center, involving improving physical facilities and using a different donor screening system, which led to increased production, yet stabilized reaction rate.

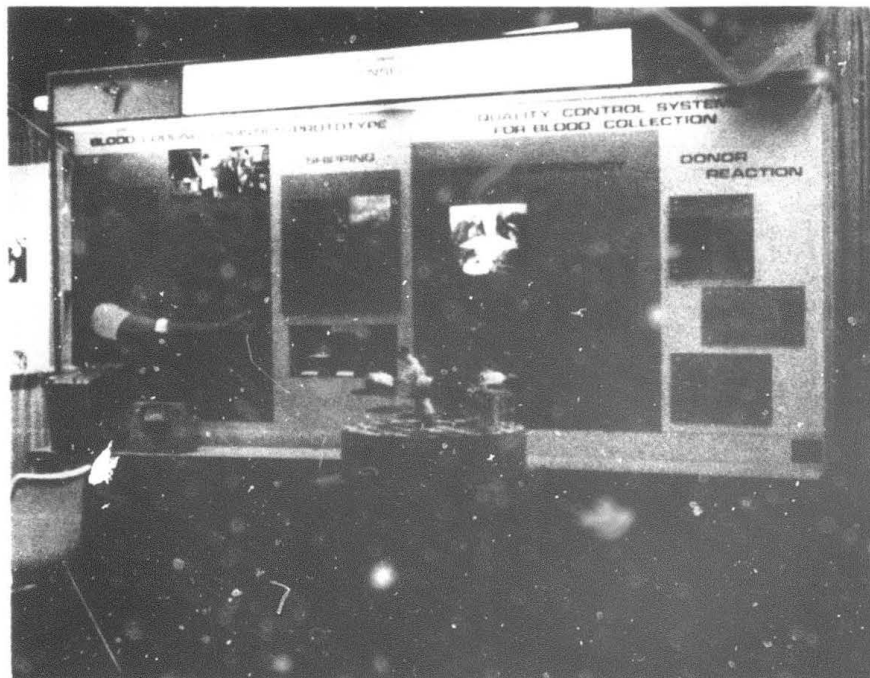


Fig. 9. Left: Blood cooling logistics--prototype systems; Right: Quality control systems applications in blood collecting operations.

1970

A Fail-Safe Approach to Incompatible Blood Transfusion (Figure 10)

LTC FRANK R. CAMP, JR., ROBERT M. NALPANDIAN, M.D., COL NICHOLAS F. CONTE, DALE L. KESSLER, M.D., LTC CHARLES E. SHIELDS, US Army Medical Research Laboratory, Fort Knox, Kentucky, and Blodgett Memorial Hospital, Grand Rapids, Michigan.

This exhibit illustrated the delegation of responsibility, with coordinated instructions, to each member of the medical team concerned with handling the detection and treatment of incompatible blood transfusions. The medical team consists of the following: nurse, clinician, anesthesiologist, and laboratory personnel. Thus, with each member complying with instructions specific to his role only, a composite, efficient, and synchronous effort is effected to accommodate all types of blood transfusion reactions, and especially to detect and salvage Incompatible Blood Transfusions (IBT). The principle of cross-check was emphasized in the exhibit with illustrations concerning the need for rapid laboratory test procedures. Finally, the Hospital Transfusion Board's role in implementing and regulating this hospital-wide detection and salvage system for victims of IBT is placed in proper perspective.

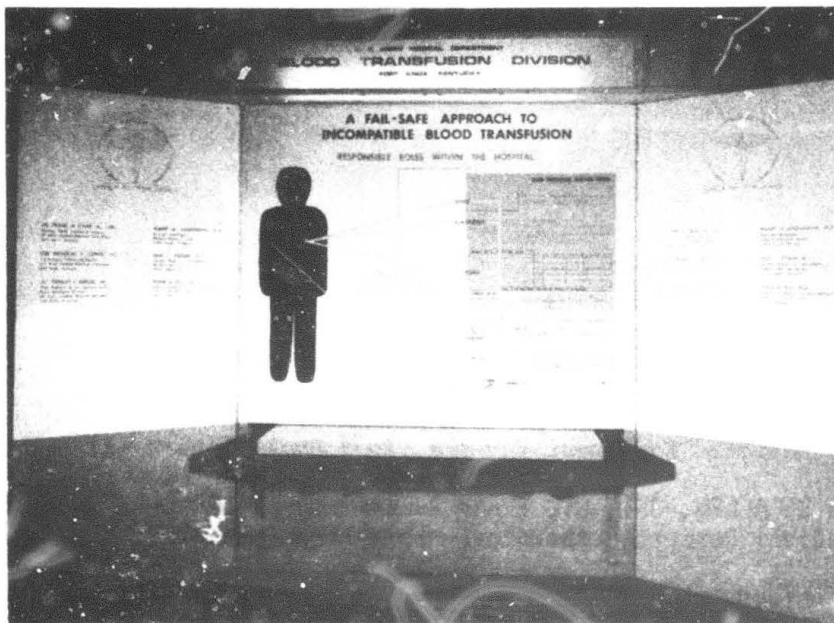


Fig. 10. A fail-safe approach to incompatible blood transfusion.

Hemoglobin Function in Stored Blood (Figure 11)

R. BEN DAWSON, JR., THOMAS J. ELLIS, CLARENCE E. SWIGGINS, DICKIE W. SPURLOCK, JAMES WILLIAMS, US Army Medical Research Laboratory, Fort Knox, Kentucky.

As 2,3-diphosphoglycerate (2,3-DPG) is depleted during storage, the left shift in the oxyhemoglobin dissociation curve develops. The relation of this defect in stored blood to oxygen transport in the recipient and ways to correct the curve to normal was illustrated.

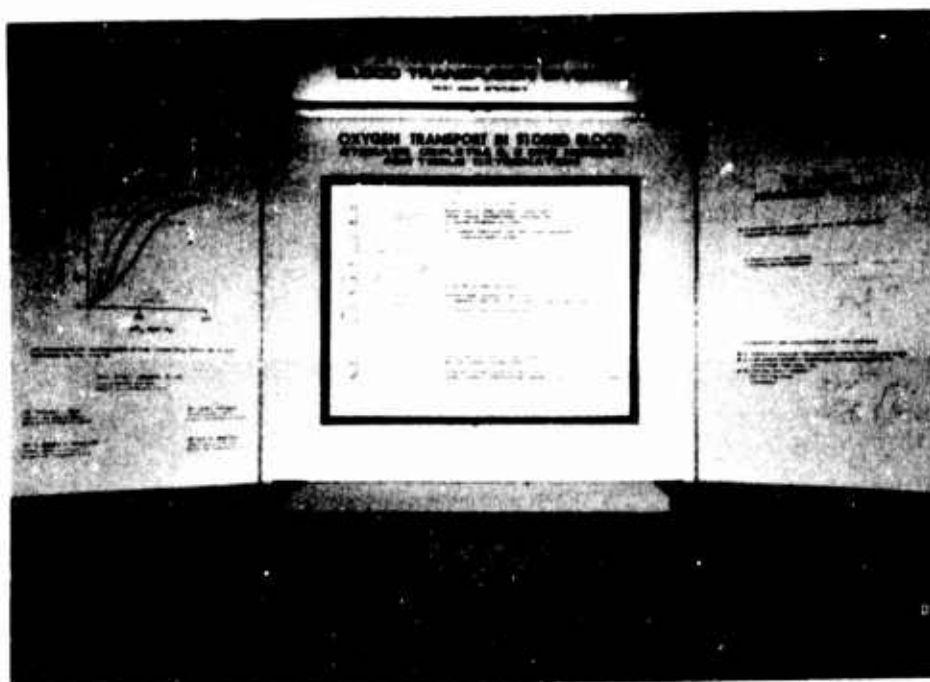


Fig. 11. Hemoglobin function in stored blood.

Newly Designed Equipment for the Blood Bank (Figure 12)

LTC TURMAN E. ALLEN, JR., LTC FRANK R. CAMP, JR., LTC CHARLES E. SHIELDS, US Army Medical Research Laboratory, Fort Knox, Kentucky.

A new blood bank device was demonstrated which was developed to increase the precision of reading test tube hemagglutination by allowing the direct and continuous observation of the red cell buttons under controlled agitation. One to five unknown cell reaction mixtures and a

negative cell control are centrifuged and then subjected to controlled agitation by the device. The unknown are compared directly with the negative control at different stages of agitation. The instrument should prove useful in detecting the presence of agglutination, in reading titers, and in antibody identification.

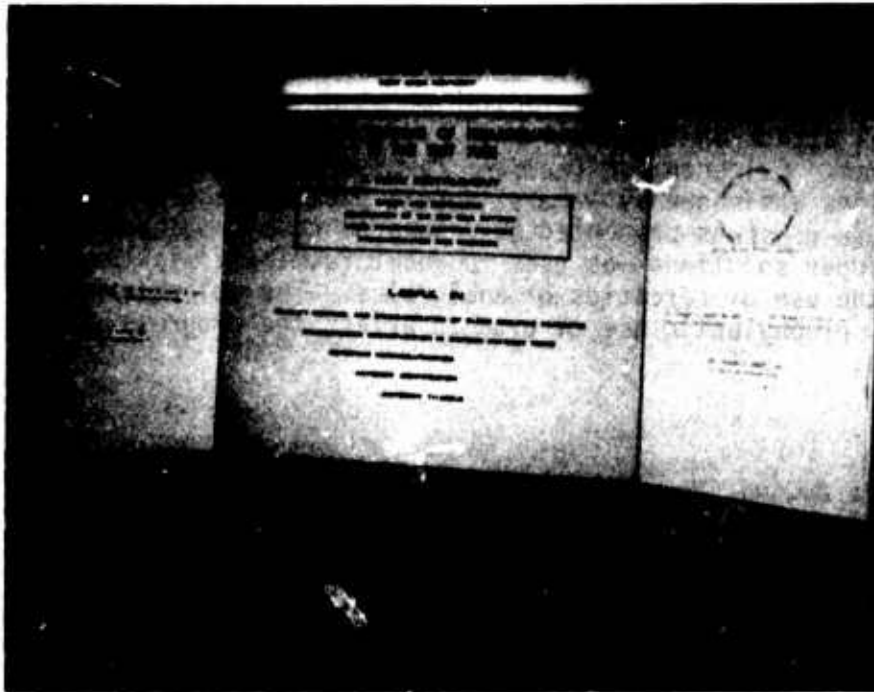


Fig. 12. Newly designed equipment for the blood bank.

1971

Research on Packaging, Cargo Coding, and Medical Product Transportation. Functional Applications for Human Biologics and Their Derivatives

DAILEY W. MC PEAK, ARTHUR J. SHULTHISE, M.D., LTC FRANK R. CAMP, JR., COL NICHOLAS F. CONTE, US Army Medical Research Laboratory, Fort Knox, Kentucky.

The specialized packaging capability and movement of human biologics and the derivatives thereof have presented multiple problems which necessitate continuing research endeavors for the improvement of the end product (blood components). This is especially important due to the dramatic increase, challenge, and current trend of blood components and blood component therapy in medical and paramedical fields. The critical requirements and sophisticated hardware necessary to accommodate the transport of blood components and derivatives were illustrated.

Molecular Basis for Sickling and the Therapeutic Use of Urea

MAKIO MURAYAMA, Ph.D., ROBERT M. NALBANDIAN, M.D., LTC FRANK R. CAMP, JR.,
COL NICHOLAS F. CONTE, US Army Medical Research Laboratory, Fort Knox,
Kentucky.

The exhibit showed the molecular basis for the mechanism of sickling red blood cells with hemoglobin S. The mechanisms were illustrated by diagrams and physical models. As an extension of the molecular hypothesis of sickling, the deductive selection of urea as the chemical desickling agent was shown. The molecular strategy for desickling by mounting a chemical attack on the implicated hydrophobic bonds was demonstrated by diagrams. Evidence for *in vitro* reversal of sickling by urea in sugar solutions was shown by Nomarski-optic photomicrographs. Additional evidence *in vivo* was presented by graphs of case summaries, in which i.v. urea in sugar solutions was used to abort fulminant sickle cell crisis without the use of narcotics or analgesics. The molecular basis for the oral, prophylactic use of urea to arrest the progress of the disease was shown.

1972

Cargo Coding "Hang Ups" in Transporting Blood and Blood Components
(Figure 13)

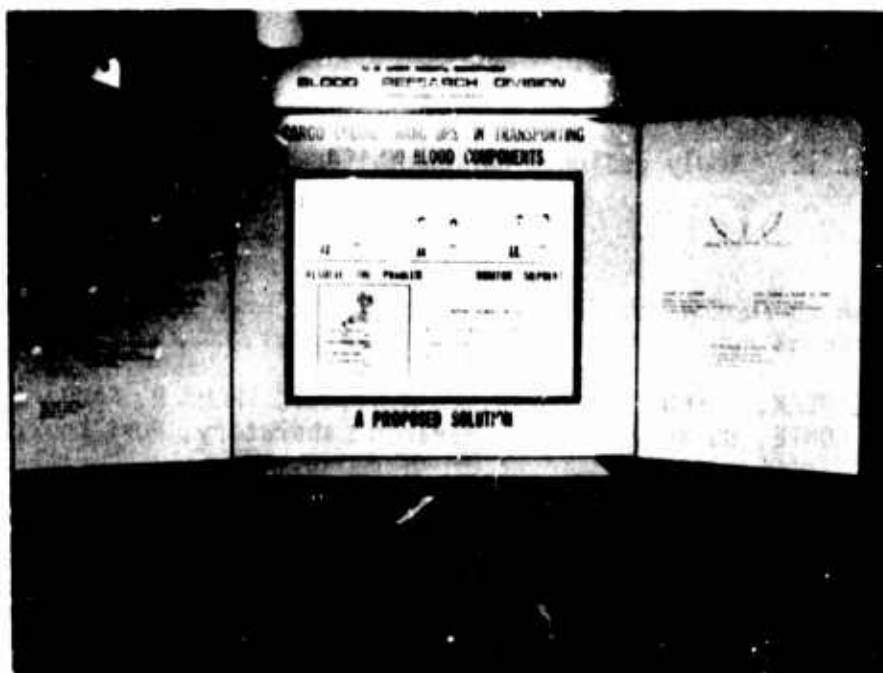


Fig. 13. Cargo coding "hang ups" in transporting blood and blood components.

DAILEY W. MC PEAK, LTC FRANK R. CAMP, JR., COL NICHOLAS F. CONTE, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Routine and emergency shipments of blood and blood products are being handled in much the same manner as regular freight was more than three decades ago. Critical requirements necessary to provide adequate protection were illustrated.

1973

Fail-Safe Refrigeration During Periods of Power Failure (Figure 14)

DAILEY W. MC PEAK, COL FRANK R. CAMP, JR., US Army Medical Research Laboratory, Fort Knox, Kentucky.

Exhibit displayed graphically and mechanically an approach to cope with the various problems associated with the loss of refrigerants used to store and transport blood and blood components.

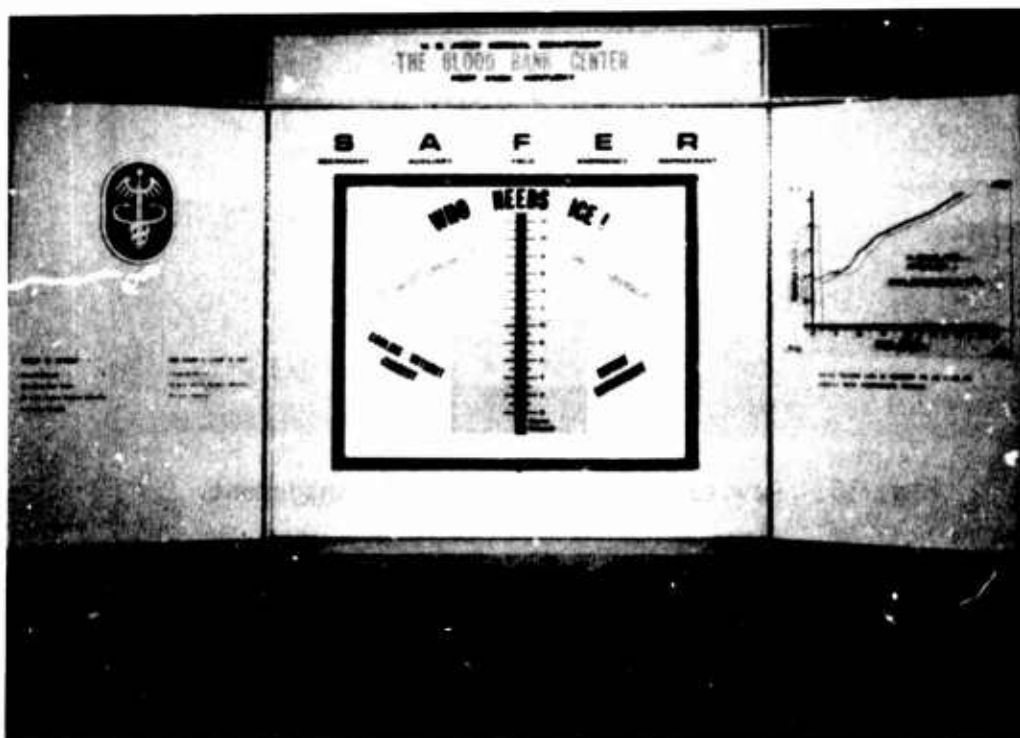


Fig. 14. Fail-safe refrigeration during periods of power failure.

Revitalization in Red Cell Management (Figure 15)

LTC BILLY W. EVANS, 1LT STANLEY C. ROBERTS, CPT PATRICIA S. SEPULVEDA,
SP5 DENNIS T. REUSS, US Army Medical Research Laboratory, Fort Knox,
Kentucky.

New concepts in blood preservation can achieve maximum utilization of donated blood. Extensive component preparation and the long-term storage of frozen red blood cells can be used to reduce blood wastage. When a unit does reach expiration, it can be biochemically regenerated and transfused.



Fig. 15. Revitalization in red cell management.

Computerized Inventory Management for Frozen Red Cells (Figure 16)

LTC BILLY W. EVANS, 1LT STANLEY C. ROBERTS, CPT PATRICIA S. SEPULVEDA,
SP5 DENNIS T. REUSS, US Army Medical Research Laboratory, Fort Knox,
Kentucky.

The computer, as applied to the needs of low temperature blood preservation, has greatly facilitated inventory management. A large

number of units can be stored and identified by the computer in a matter of seconds. The computer is effective in keeping transfusion records and shipping data, virtually eliminating human error in transcription.

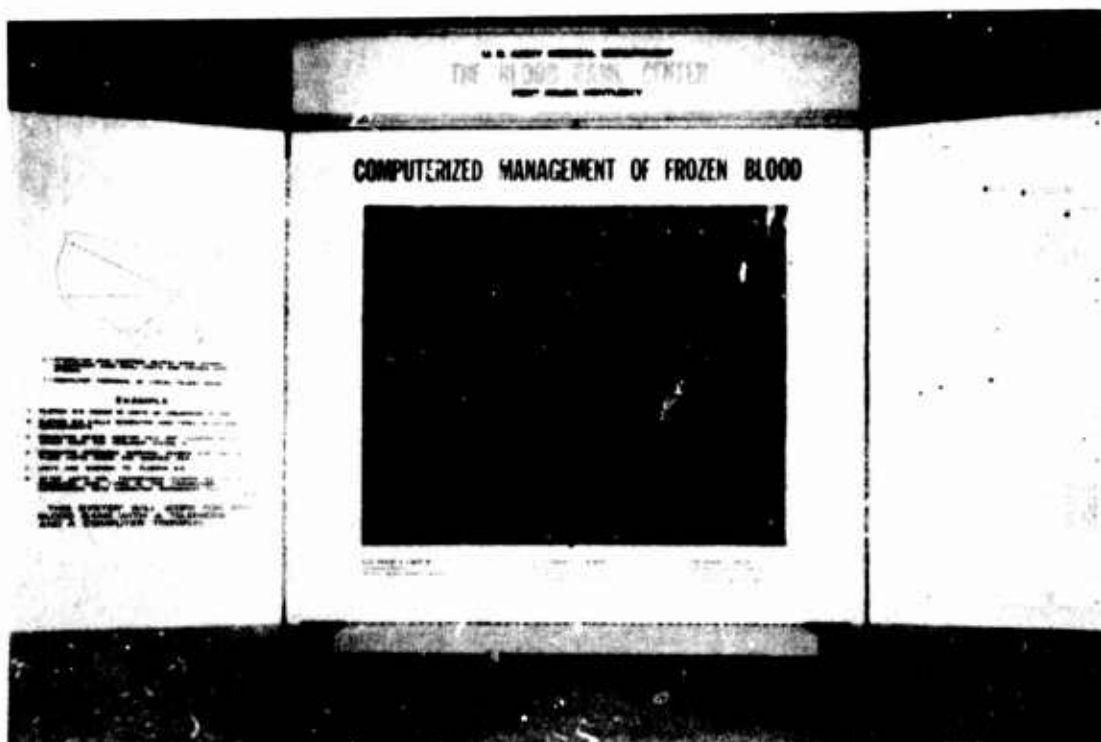


Fig. 16. Computerized inventory management for frozen red cells.

Partners in Education (Figure 17)

COL FRANK R. CAMP, JR., WILLIAM D. HANN, Ph.D., COL CHARLES E. SHIELDS, MAJ VIRGIL R. COLEY, CPT KENNETH I. TOBIAS, LTC JERRY R. BREWER, CPT PATRICIA S. SEPULVEDA, US Army Medical Research Laboratory, Fort Knox, Kentucky.

The exhibit shows the programs resulting from the affiliation of the US Army Medical Research Laboratory and the Bowling Green State

University, Ohio. The goal of the program and exhibit was to point the way for educated technologists equipped with graduate education beyond the Masters degree to deliver quality health care.

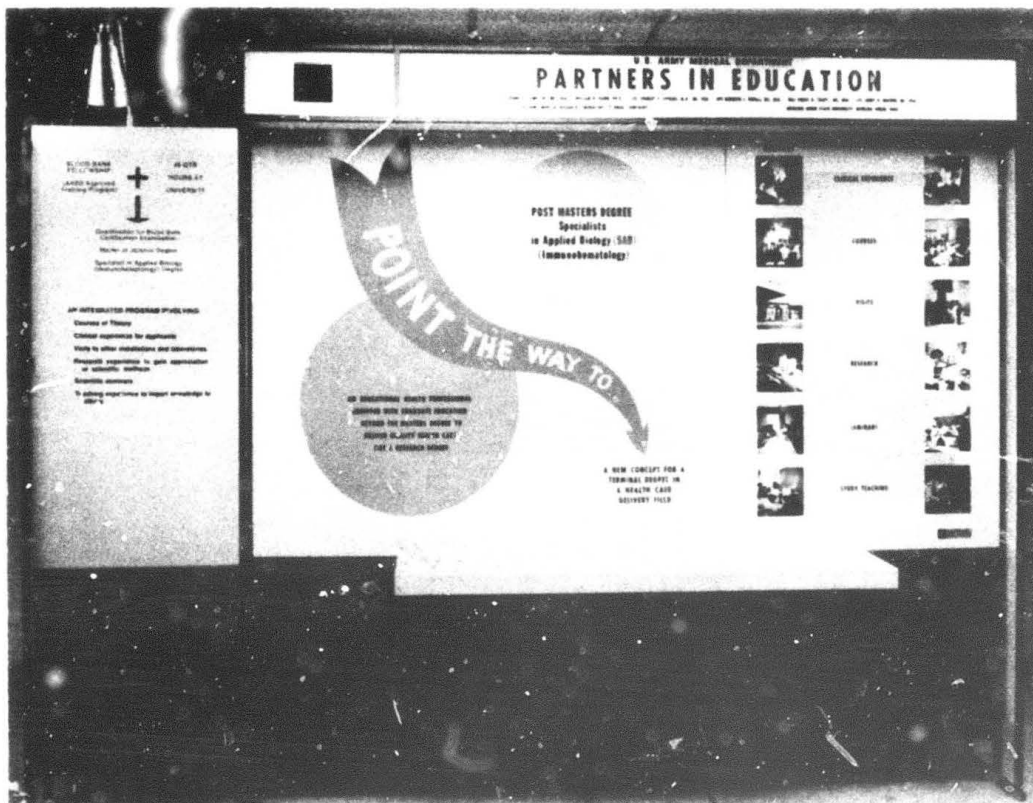


Fig. 17. Partners in education.

Oxygen Transport in Dogs (Figure 18)

COL CHARLES E. SHIELDS, CPT MARK G. BURNS, ANGELO I. ZEGNA, SP6 DAVID E. MEIXNER, US Army Medical Research Laboratory, Fort Knox, Kentucky.

Effect on the oxygen transport of 50% acute hemorrhage in a dog model was studied. Blood stored with and without adenine was evaluated and the decrease in p50 and DPG which caused a decrease and then recovery in the recipient, was observed.

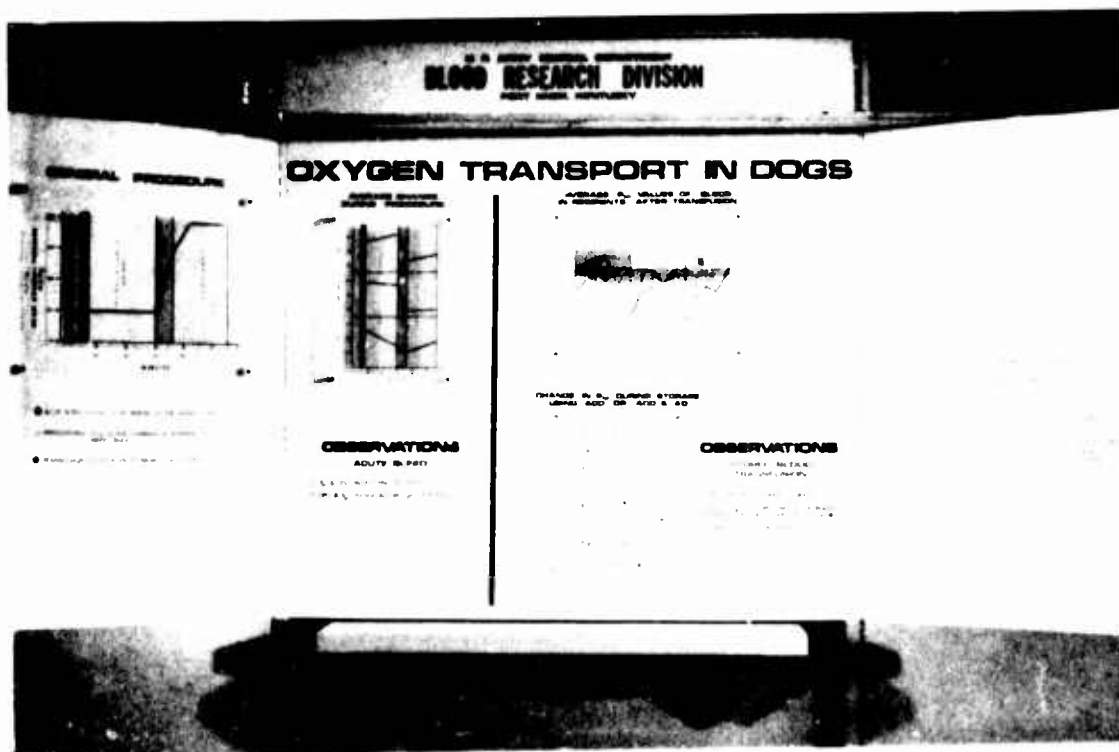


Fig. 18. Oxygen transport in dogs.

A N N E X U

A COLLECTED BIBLIOGRAPHY OF CLINICAL ADVANCES
IN SICKLE CELL DISEASE BASED ON
THE MURAYAMA MOLECULAR HYPOTHESIS
(Updated from 25 February 1972)

AD _____

REPORT NO. 1,009

A COLLECTED BIBLIOGRAPHY OF CLINICAL ADVANCES
IN SICKLE CELL DISEASE BASED ON
THE MURAYAMA MOLECULAR HYPOTHESIS
(Updated from 25 February 1972)

(Progress Report)

by

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16 November 1972

Military Blood Banking:
Methodology and Equipment
Work Unit No. 158
Combat Surgery
Task No. 00
Combat Surgery
DA Project No. 3A062110A821

Approved for public release; distribution unlimited.

ABSTRACT

A COLLECTED BIBLIOGRAPHY OF CLINICAL ADVANCES
IN SICKLE CELL DISEASE BASED ON
THE MURAYAMA MOLECULAR HYPOTHESIS
(Updated from 25 February 1972)

OBJECTIVE

To collect and make conveniently available in one source a bibliography of all scientific communications dealing with diagnostic and therapeutic advances in sickle cell disease derived from the Murayama hypothesis for the molecular events involved in the sickling of a hemoglobin S red cell. This bibliography has been updated from that published 25 February 1972, in USAMRL Report No. 966.

METHOD

The published papers, abstracts, presentations at national and international scientific meetings, books, a scientific exhibit, a television documentary, and other scientific communications are collected and arranged in the order of publication or presentation. Listed in this bibliography are 105 such items. The cited research work emanates principally from three institutions: Department of Pathology, Blodgett Memorial Hospital, Grand Rapids, Michigan; Department of Physiology, School of Medicine, Wayne State University, Detroit, Michigan; and the US Army Medical Research Laboratory, Fort Knox, Kentucky.

CONCLUSIONS

The Murayama hypothesis for the molecular events involved in the interior of a sickling hemoglobin red cell is based in part on his construction of a precision scale model of the hemoglobin S molecule. Murayama, drawing in part upon the molecular investigations of others, such as Pauling, Braunitzer, Konigsberg, Muirhead, Perutz, and Ingram, has in effect provided us with a molecular definition of disease and a description of molecular interaction which has pathogenetic significance in sickle cell disease. This is the first time ANY disease has been understood in such terms at the molecular level in medicine. The cited references in this bibliography are all directly derived from the Murayama hypothesis either by deduction or inference. These scientific communications represent advances at the clinical level. Our group has bridged the growing gap between advancing sophisticated basic science work and an effective transliteration of such information into clinical gains in sickle cell disease. All of the following discoveries are such extensions of the Murayama hypothesis:

1. Five inexpensive, new tests for hemoglobin S, each with a molecular basis: (a) Murayama test; (b) four dithionite tests.

2. Several successful mass screening studies for sickle cell hemoglobin in large military and civilian populations by the automated dithionite test.

3. A therapeutic molecular strategy for mounting a chemical attack on the intertetrameric hydrophobic bonds essential to the sickling event.

4. The theoretical specifications defining an effective desickling agent including some particulars of molecular structure which ANTEDATED and led to the selection of urea.

5. Optical and electron microscopy evidence for the desickling effect of urea.

6. The evolution of a safe and effective clinical protocol for the intravenous use of urea in sugar solutions in the treatment of acute sickle cell crisis without the effect of narcotics or analgesics.

7. The evolution of a simple clinical protocol for the oral, prophylactic treatment of sickle cell disease and related clinical conditions.

Each and every time a prediction was made from the Murayama hypothesis experimental results confirming the deduction or inference were consistently forthcoming. Without too much exaggeration, one may view the Murayama hypothesis as a scientific cornucopia of innovative clinical advances in sickle cell disease.

It is for the convenience of the serious student of sickle cell disease who may wish to advance the clinical knowledge of sickle cell disease by the *molecular route* of the Murayama hypothesis that we have compiled this pertinent bibliography.

The citations in this publication span only four years—from November 1969. The scientific content of these citations only begins to suggest the truth, power, and beauty of the Murayama hypothesis. It is our hope that other workers will pick up this powerful tool which Murayama has given us and will advance the clinical gains in diagnosis and treatment of sickle cell disease, a deadly if lingering affliction which numbers its world-wide victims in the untold millions.

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A COLLECTED BIBLIOGRAPHY OF CLINICAL ADVANCES
IN SICKLE CELL DISEASE BASED ON
THE MURAYAMA MOLECULAR HYPOTHESIS
(Updated from 25 February 1972)

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A N N E X V
AN AUTOMATED MILITARY BLOOD TYPING SYSTEM

REPORT NO. 1,025

AN AUTOMATED MILITARY BLOOD TYPING SYSTEM

(Progress Report)

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ABSTRACT

AN AUTOMATED MILITARY BLOOD TYPING SYSTEM

OBJECTIVE

To implement an automated military blood typing system designed to reduce the extent of the manual tasks and at the same time enhance the accuracy and reliability of the blood typing process.

MATERIALS AND METHODS

The major features of the automated military blood typing system are:

- a. Test Result Readers which will automatically read up to 15 channels of AutoAnalyzer output directly from the filter paper record currently in use.
- b. Sample Identification units which will automatically encode Vacutainers with machine and human readable numbers and which will read these numbers automatically during AutoAnalyzer testing.
- c. A means of automatically supplying blood type tags for labeling whole blood bags.
- d. Automatic generation of roster sheets for use in the preparation of recruit dog tags.
- e. Complete status and daily summary reporting of all blood typing operations.
- f. Donor/recruit information file providing rapid access to blood analysis histories.

RESULTS

In general, the automated system eliminates errors resulting from tedious manual transcription. It saves time and effort by permitting blood to be analyzed in unspecified order due to the prenumbered machine readable samples. It saves time, labor, and errors by performing automatic analysis of blood group and type. In addition, the system eliminates paper work with its donor/recruit file. Perhaps the most important, it stores vital donor/recruit information which can be recalled in a moment's notice at any time. Finally, there is generation of error-free rosters for preparation of military identification tags.

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FOREWORD

This report describes a Research and Development prototype: The Automated Military Blood Typing System for use in hospital blood banks and blood collection and processing centers. There are several requirements for pursuing this effort which relate directly to the soldier in combat and to military and civilian patients in hospitals. These include:

1. To reduce clerical and technical error in technics of blood grouping, blood banking, and blood transfusion.
2. To eliminate injury and death to patients receiving blood transfusions.
3. To provide rapid and accurate information to the Hospital Transfusion Board for determination of the cause of a transfusion accident and to prevent further accidents.
4. To provide a reliable data base for research analysis.

PREFACE

Historic Background of Blood Grouping

Modern concepts of medical care demand an ever increasing volume of blood safe for transfusion. In striving to meet this demand, one finds no less responsibility imposed upon the military for efficient blood grouping procedures than has been placed upon the civilian medical facility. The term mass blood grouping generally refers to the testing of unknown cells with sera of known antibody content and the verification of this testing by determining which antibodies are present in the serum of the sample of blood. Additional tests are included for the Rh factor, immune antibodies, and, finally, reliable controls are employed. Reactions of such testing are usually read by eye and only the practice-acquired personal experience of a technician can minimize variables in reading results (1).

An example of large-scale routine blood typing procedures used in the military nearly a decade ago is shown in Figure 1, illustrating the assembly line approach to manually group, back-type, and titer 3000 group O bloods. Some rapidity was obtained by using wooden applicator

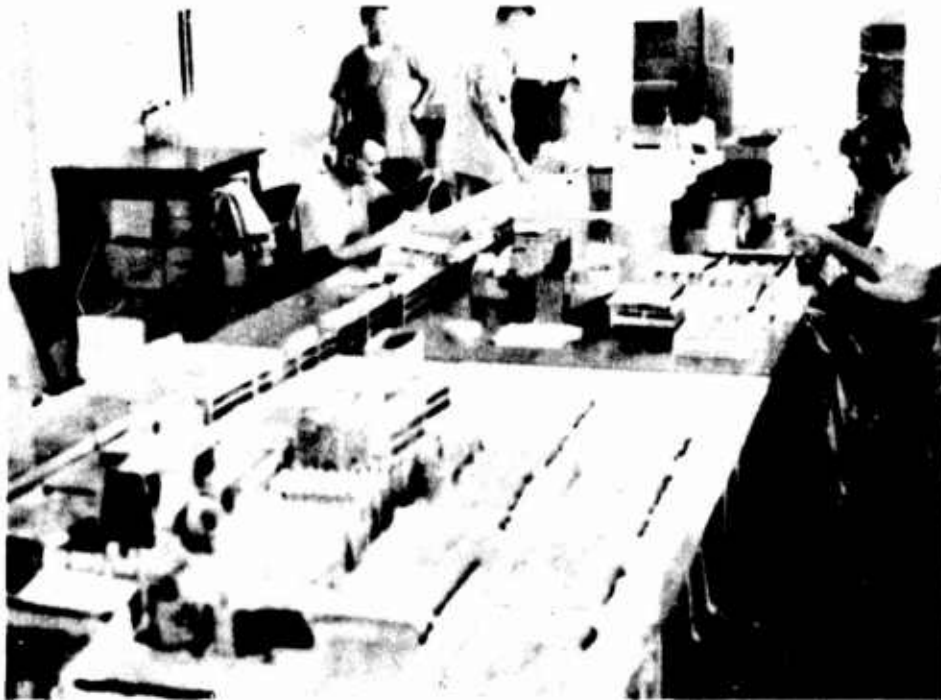


Fig. 1. Thousands of manually performed tests involved in a field study of blood groups of troops of the 1st Cavalry Division, 1965.

sticks referred to frequently as the dip stick technic of adding red cells. Figure 2 shows the employment of semiautomation for titering natural anti-A and anti-B antibodies with an accurately calibrated loop (2). The loop delivered 0.01 ml of serum to each tube used for preparing

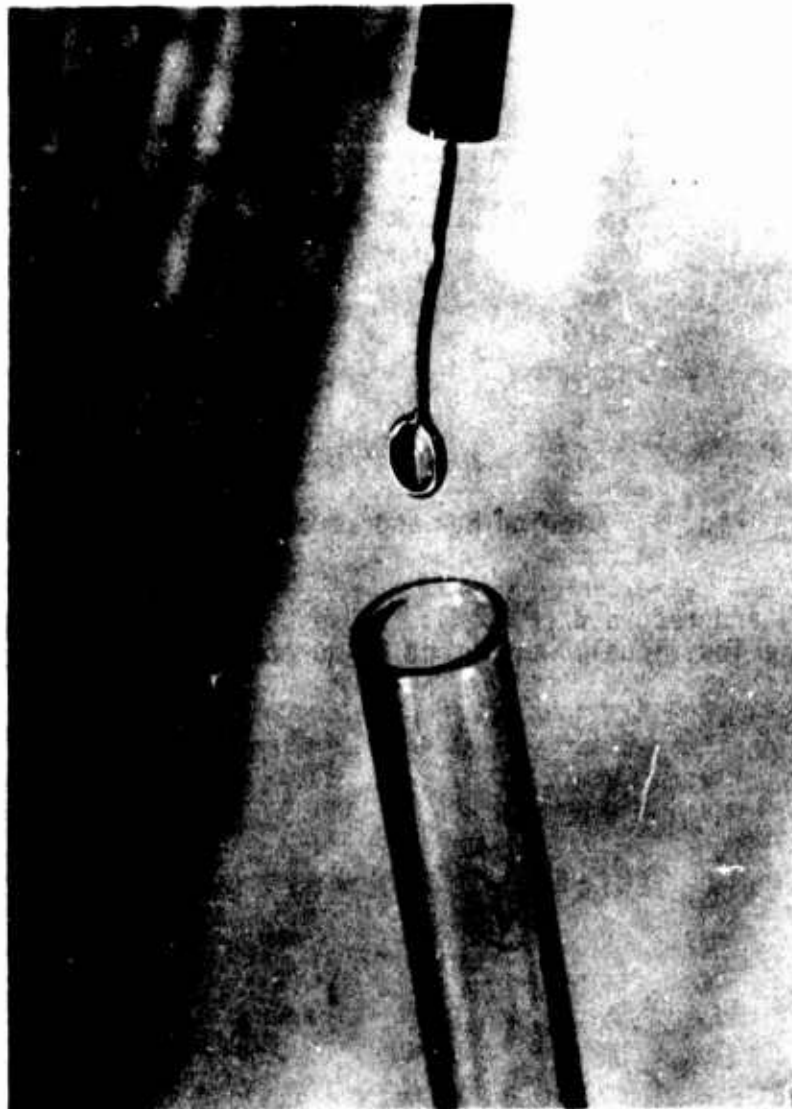


Fig. 2. Calibrated loop for rapid delivery of serum for titering natural anti-A and anti-B antibodies.

a 1:200 dilution of serum in saline. Following all of these procedures and centrifugation of thousands of 12 x 75 mm test tubes, another critical step remained as shown in Figure 3. Each reaction had to be read,

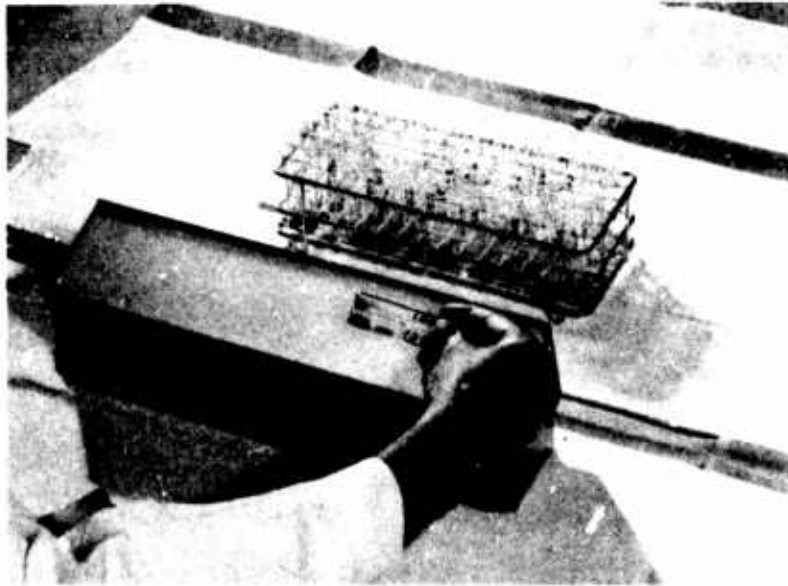


Fig. 3. Viewing box and serologic reactions.

interpreted, and recorded (Fig. 4). These bloods were screened to confirm that the individuals were group 0 and could be used, if necessary,



Fig. 4. Reading and recording results of serologic testing.

as Universal Donors for A, B, and AB recipients. Hemolysins were also recorded. It is interesting to note that bloods classified as having anti-A and anti-B serum titers of less than 1:200 were later shown to be accurate by comparison with the twofold serial dilution technic employing highly precise quantitative pipettes and procedure. The following quotation (8) is included to emphasize the fact that workers clearly recognized the problem and requirement for automated detection of hemagglutination reactions in 1965-66. Some of these and other workers appreciated the need for automation in the field of immunohematology even earlier.

"Mass blood screening has the major problem of mechanical manipulation and identification of blood samples to be analyzed. There has been a general lack of technics and instrumentation which would allow fast and yet precise measurements of hemagglutination with small samples. The desirable solution to this problem is a system which will allow the major routine analyses to be performed on small samples of blood which are drawn and contained within disposable containers which are thereafter automatically manipulated and identified. Significant improvements in the state of the art have been made available through the tremendous instrumentation and automation technology which has been developed for other fields, such as nuclear physics and molecular biology research (3).

"The need for automated detection of hemagglutination reactions has been recognized for some time, and in 1965 Sturgeon, McQuiston, DuCros, and Smythe reported on advances with the autoanalyzer for automated blood typing (4,5). Also in 1965, Rosenfield and Haber reported on the 'detection and measurement of homologous human hemagglutinins' (6). Other reports are listed in the references (7-13)."

Historic Background of The Blood Bank Center, Fort Knox, Kentucky (17-32)

The Blood Bank Center at Fort Knox, Kentucky, processes a large number of blood samples daily. In 1965, when the Center was created, manual technics were employed exclusively (Fig. 5). By 1966, a Technicon Blood Typing AutoAnalyzer was put into operation and the results collated with manual testing (Fig. 6). During the period 1966-68, the manual and automated systems were compared in the processing laboratory (Fig. 6a).

An 8-channel AutoAnalyzer was used to identify A-B-O blood groups and Rh types of recruit soldiers, and a 15-channel analyzer performed the blood group serology required for labeling units of whole blood in the blood bank. Error on military identification tags, prepared from AutoAnalyzer blood group results, was less than 1%, resulting from both technical and clerical error. Manual testing, performed at various Army laboratories throughout the country, resulted in an error rate of 8%. This A-B-O and Rh error rate on identification tags, prepared from manual data, is also a combination of technical and clerical errors.



Fig. 5. During 1965-66 all of the serologic testing of the Fort Knox Blood Bank Center consisted solely of manual technics.

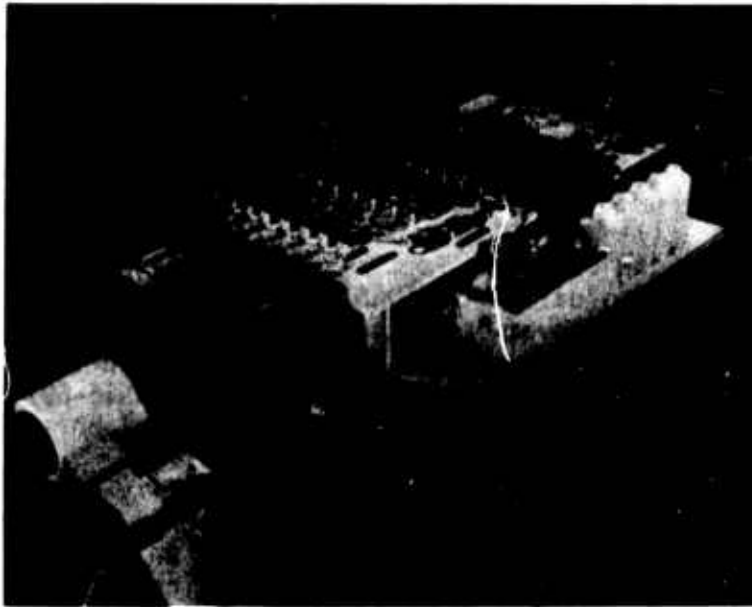


Fig. 6. A Technicon Blood Typing AutoAnalyzer was placed in operation during 1966 and compared with manual testing.



Fig. 6a. Comparison of manual and AutoAnalyzer results was conducted during 1966-68. Error on dog tags was reduced to less than 1% using AutoAnalyzer results for ABO and Rh.

Based on this experience, it was apparent that the blood group AutoAnalyzer was a step in the right direction. On the other hand, there still remained an inordinate number of manually performed tasks related to both the instrumentation and the handling and processing of the large amounts of data that are generated daily. Since 1968, serious discussion and planning have been conducted with appropriate persons in government agencies, directors and technologists of blood bank centers and hospital blood banks, and, finally, with engineers in industry.

By 1970, the Automated Military Blood Typing System was proposed. It was designed to reduce the extent of blood bank manual activities associated with the blood group AutoAnalyzer as we know it today, and, at the same time, to enhance the accuracy and reliability of the blood typing process.

We have seen that automation, in the form of the AutoAnalyzer, has contributed significantly to the reduction of the error rate in the blood typing process, but even an error rate of less than 1% is undesirable and must be eliminated. The large majority of these remaining errors can be attributed to the lack of a system which automatically provides positive sample identification throughout the blood testing and reporting process. The Automated Military Blood Typing System is designed to overcome this deficiency and will eliminate the error-prone manual activities associated with the identification of samples, the interpretation of typing data, and the reporting of test results.

AN AUTOMATED MILITARY BLOOD TYPING SYSTEM*

INTRODUCTION (14-16)

Rapid and accurate blood typing of donors and recruits is essential to conserve the fighting strength of the combat soldier. Efficient and reliable record keeping and data processing are necessary to provide the rapid source of information required to adequately support the blood needs of a modern army. The use of Technicon Blood Typing AutoAnalyzers at major blood banks has permitted the rapid processing of large numbers of blood samples, but an inordinate amount of manual tasks related both to instrumentation, handling, and data processing of large numbers of blood samples still remain.

The Automated Military Blood Typing System is under development through the US Army Medical Research and Development Command and the US Army Medical Research Laboratory, Fort Knox, Kentucky, by American Science and Engineering, Inc., Cambridge, Massachusetts. This system is designed to reduce the extent of the manual tasks and at the same time enhance the accuracy and reliability of the blood typing process.

The system is transportable and easily packaged into mobile hospital units. It can be placed in key central areas, and remote terminals can directly assess the blood information files from field locations, obtaining critical medical information instantly in emergency situations. The system is readily expandable to provide inventory control and complete blood bank management.

MATERIALS AND METHODS

System Components

Figure 7 shows the blood collection kits, consisting of blood packs, Vacutainers, and donor registration forms being assembled at the blood bank. Vacutainers are placed in the Vacutainer Writer Unit, and a blood number, up to six digits, is written on the Vacutainers in both machine and human readable form. The same number is simultaneously printed on ordinary gummed labels which are then attached to the plastic blood bag and to a donor registration form.

Figure 8 shows a typical Vacutainer bearing a permanent label on which machine and human readable information has been written. The sample identification system consists of Vacutainer Writers, used at the time of blood collection, and Vacutainer Readers, which read the blood number during AutoAnalyzer testing.

* Presented, in part, by COL Frank R. Camp, Jr., MSC, at the Transfusion Congress/AABB, Washington, D. C., 27 August - 2 September 1972.

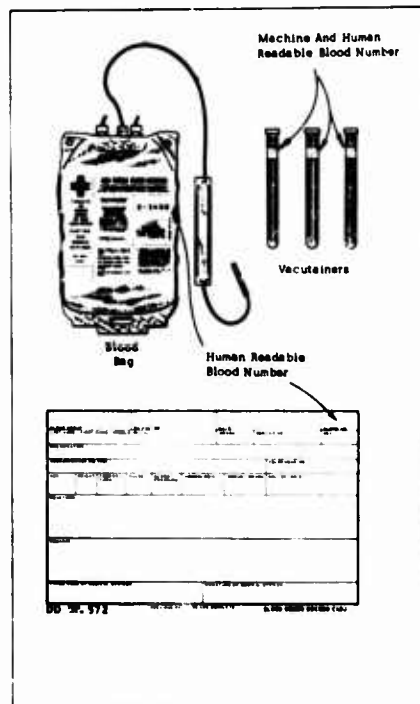


Fig. 7. Blood collection kit.



Fig. 8. Vacutainer.

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Test Results

A Test Result Reader has been designed, fabricated, and tested. Figure 9 is a photograph of this device. The output from the AutoAnalyzer consists of a stream of continuous filter paper with rows of agglutination deposits. This paper is inserted into a paper drive mechanism which advances the continuous filter paper from the AutoAnalyzer past an array of photo diodes. A lamp illuminating the photo diodes is differentially attenuated by the passage of "positive" and "negative" blood agglutination deposits between the light source and the diode detector. Light transmittance information is digitized in the Test Result Reader by an analog-to-digital converter, and passed to a minicomputer for data processing. The computer analyzes the light transmittance information and computes the blood type of the sample. This information is then transmitted back to a computer-controlled printer in the Test Result Reader. This device prints the correct blood type next to the agglutination deposits, providing a permanent copy of all blood typing results. The system will also print the blood number above each blood type.

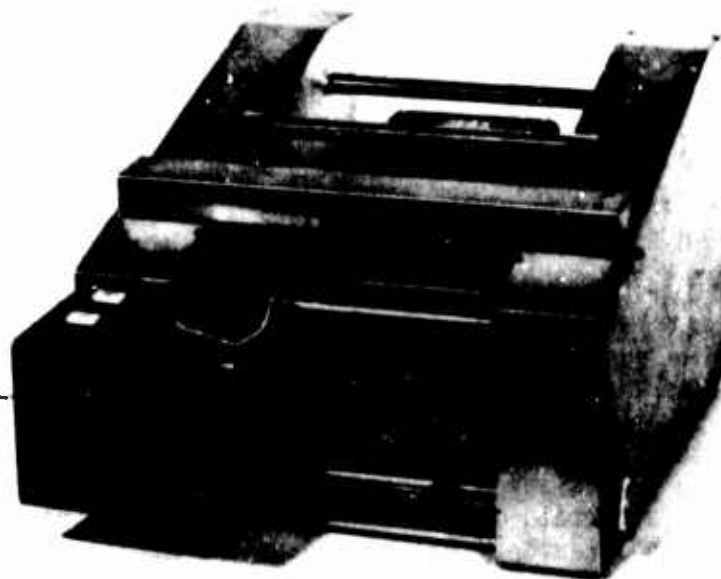


Fig. 9. Automated Test Result Reader.

Figure 10 shows a section of filter paper analyzed by present procedures. An operator has manually analyzed the agglutination deposit patterns and marked each respective blood type with a marking pencil.

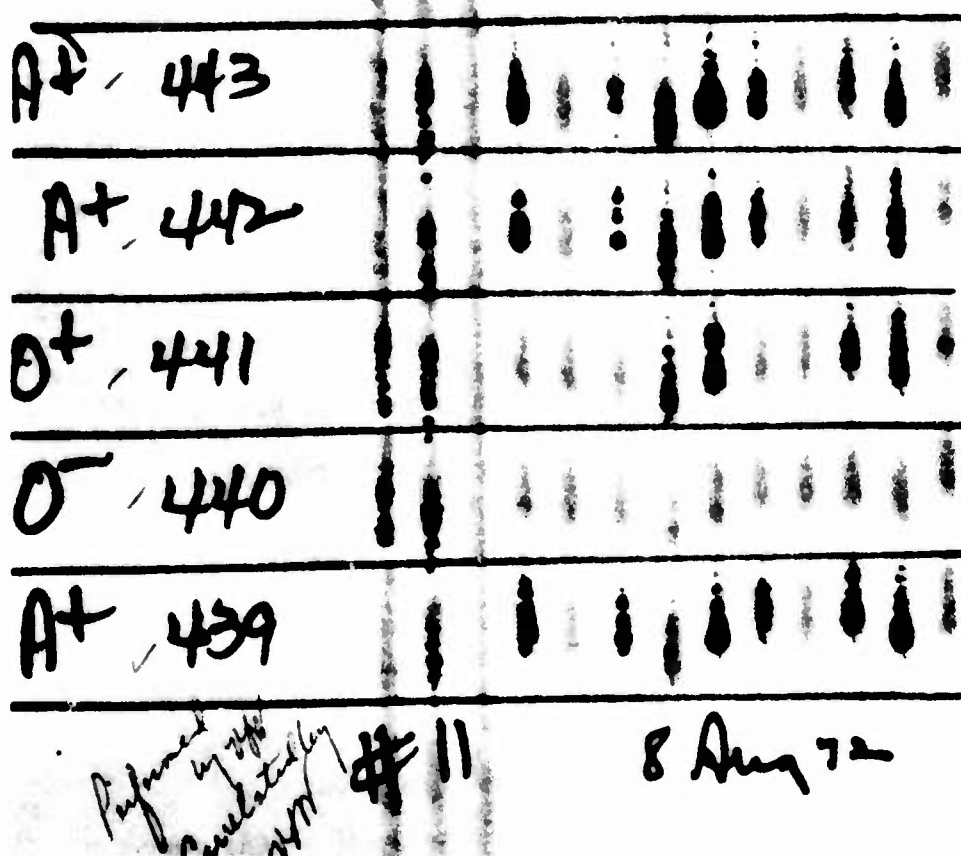


Fig. 10. Agglutination deposits which have been analyzed and manually recorded.

Figure 11 shows a section of filter paper analyzed by the Test Result Reader. Blood type and sequential accession number have been automatically entered by the computer-controlled printer. The Test Result Reader is designed both to detect the occurrence of any deposit (be it positive or negative) and to ascertain whether the result is positive, negative, or ambiguous. For this reason, the phasing of the AutoAnalyzer channels is not critical, since a computer does not have to "look" at the filter paper at any particular time, but, rather, is notified (interrupted) by the Test Result Reader that a test result is ready for transfer. When the computer is interrupted, it strobes the test result data into memory and performs a series of tests to determine the measured blood group. A printer attached to the Test Result Reader prints the sample identification (blood number) and blood group directly on the moving filter paper adjacent to the agglutinations to which they correspond. This allows manual verification of test results at any time.

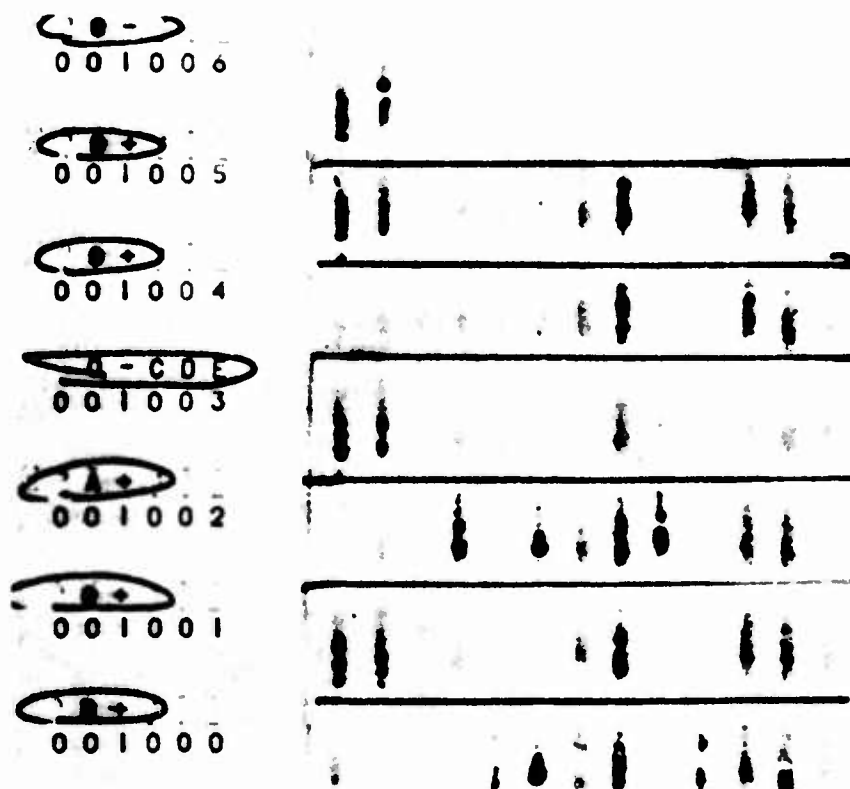


Fig. 11. Analysis of filter paper deposits by test result reader. The blood type and sequential accession number have been automatically entered by the computer-controlled printer.

Data Processing

A Data General Nova 1220 minicomputer with teletype terminal constitutes the system program and performs the following tasks:

- a. Initiates the system and provides for orderly start and shut-down.
- b. Communicates with operator via teletype; transmits and receives information regarding system operation and performance.
- c. Interfaces with Test Result Reader analog-to-digital converter to receive light transmittance information.
- d. Analyzes light transmittance data using previous transmittance information, interchannel redundancy, and known system parameters to determine blood type of each sample.
- e. Stores each blood type determination until ready for print-out.

f. Stores blood numbers from Vacutainer Reader and correlates them with respective blood type.

g. Interfaces with computer-controlled printer to print out information on continuous filter paper.

h. Alerts operator via teletype of all abnormalities determined during data analysis.

A photograph of the Nova 1220 minicomputer is shown in Figure 12. The computer, which is supplied with 8,000 words of memory (16 bits per word), can be expanded to 32,000 words without modification, allowing for future system expansion.

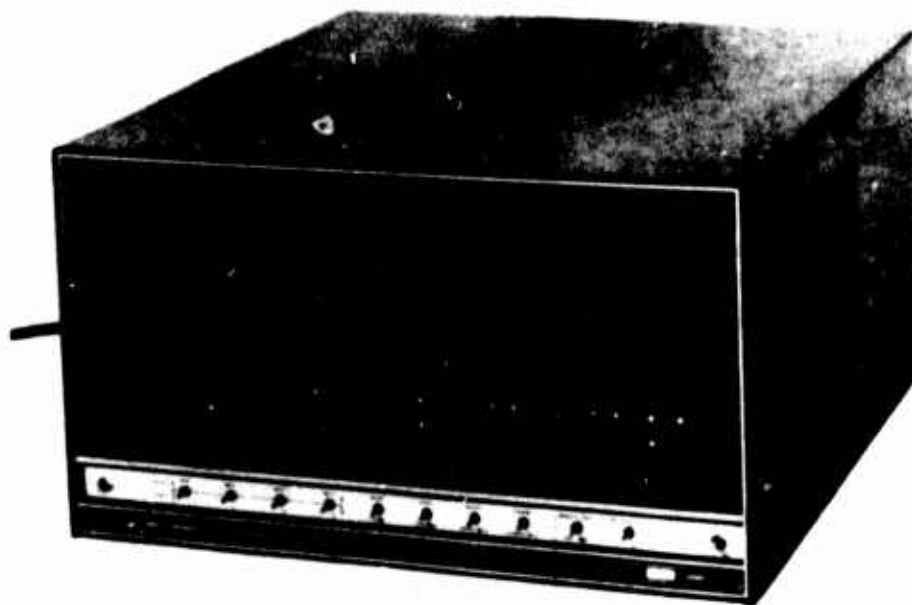


Fig. 12. Nova 1220 minicomputer.

Sample Identification

Vacutainer Writer (Fig. 13). This instrument does not tie into the minicomputer system, but is used in the blood collecting kit preparation area. When an empty Vacutainer bearing a preaffixed blank thermal sensitive label is inserted into the machine, a sample identification number is written on the label. The writing is accomplished by selective

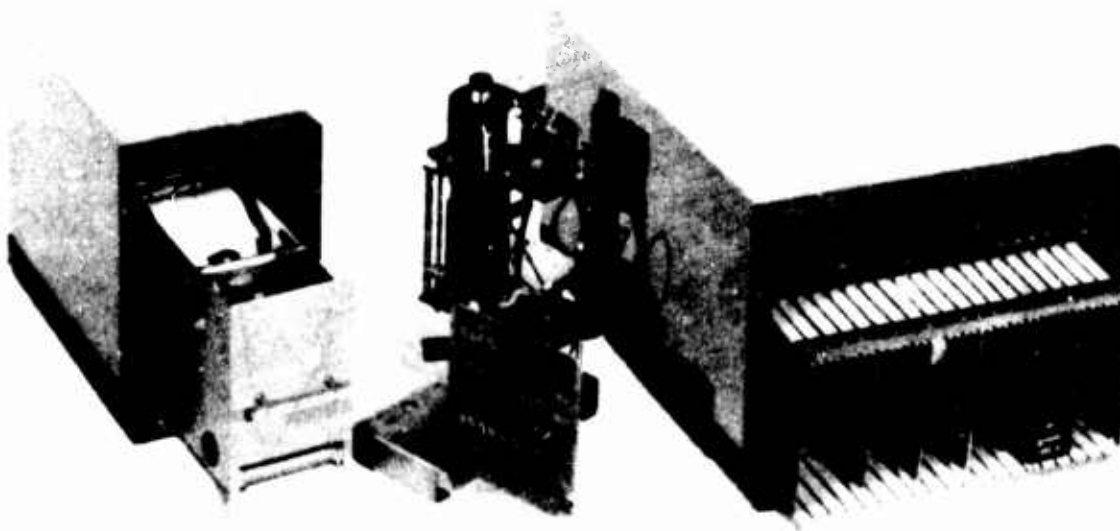


Fig. 13. Vacutainer Writer prior to assembly.

application of power to thermal elements which contact the label while the Vacutainer is rotated. The identification number is written in both machine and human readable code. The machine then reads and verifies the number and prints out a series of pressure sensitive labels (quantity selectable from 0 to 10). These labels are used to encode other articles used in the blood collection process (blood bag, donor card, additional Vacutainers for manual test requirements). After the label printing, the Vacutainer is ejected, the sample number is incremented by one, and the machine is ready to encode the next Vacutainer. The sample number is resettable to any six digit number.

Vacutainer Reader. After the encoded Vacutainer is filled with the blood sample, it is placed in the turntable of the Technicon AutoAnalyzer for testing. American Science and Engineering (AS&E) has formulated a design using a folding chain, made of injection molded plastic links. A sketch of this design is shown in Figure 14.

This approach is widely used in various sample measuring devices, including scintillation counters. The design allows a precision reading and aspirating station in which the Vacutainer is accessible from all sides. Thus, the Vacutainer can be mechanically positioned and rotated with the proper precision, resulting in accurate and dependable reading of the sample identification number.

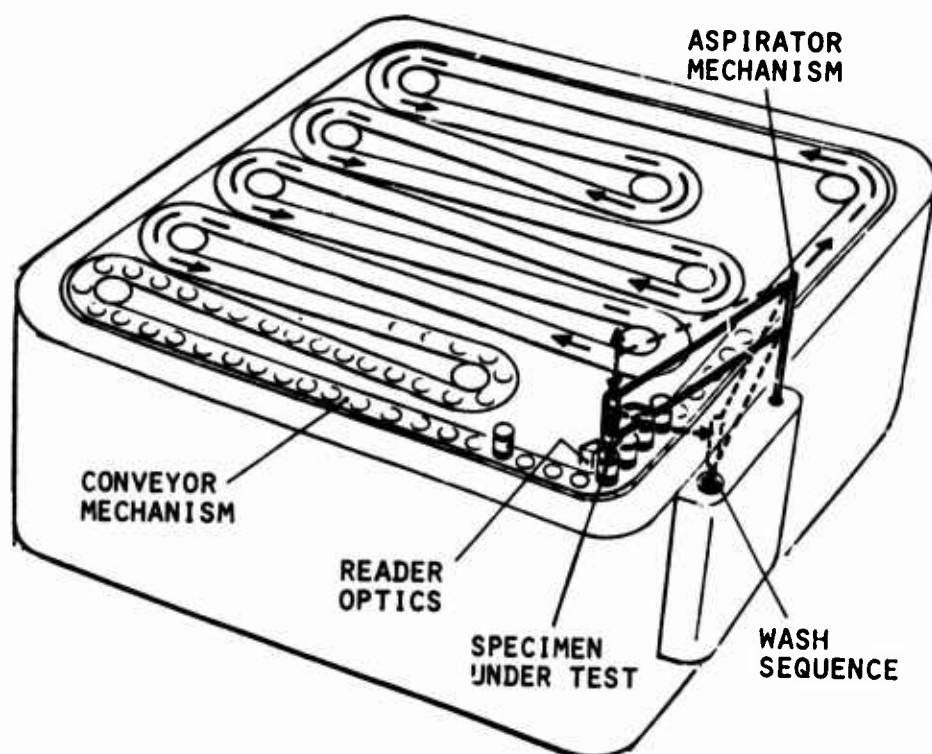


Fig. 14. Vacutainer Reader.

System Operation. The samples to be tested are loaded into the AutoAnalyzer following present procedures, and the system is switched on. At that point, the minicomputer takes command of the operation and communicates with the operator via teletype. The operator answers several questions, including date, time, etc. The computer instructs the operator to turn on the Technicon Vacutainer sampler and notify the computer. After this is accomplished, there is a time period of approximately 15 minutes before the first sample is processed by the AutoAnalyzer. At this point, there is sufficient filter paper at the output of the AutoAnalyzer to load into the Test Result Reader. The operator loads the filter paper into the paper drive mechanism of the Test Result Reader, with the first row of agglutination deposits in line with special alignment markers. The computer is then notified to start analysis. If the operator fails to perform this operation within 25 minutes after initiation, the computer reminds him to do so.

The blood group and type data are analyzed by the Test Result Reader and computer, and are then printed next to each respective row of agglutination deposits. As an added feature, a sequential list of blood types is available from the teletype. When an ambiguous blood type occurs, or one which cannot be adequately determined by the AutoAnalyzer reagents, the operator is immediately notified. These samples usually require additional manual testing in the laboratory.

To shut down the system, the operator notifies the computer and turns off the Technicon Vacutainer sampler. The system takes 15 minutes to complete analysis on the samples in various states of chemical processing. After the last sample result has been printed, the system switches off automatically.

Figure 15 shows a section of teletype output during start-up and initiation of the blood typing system.

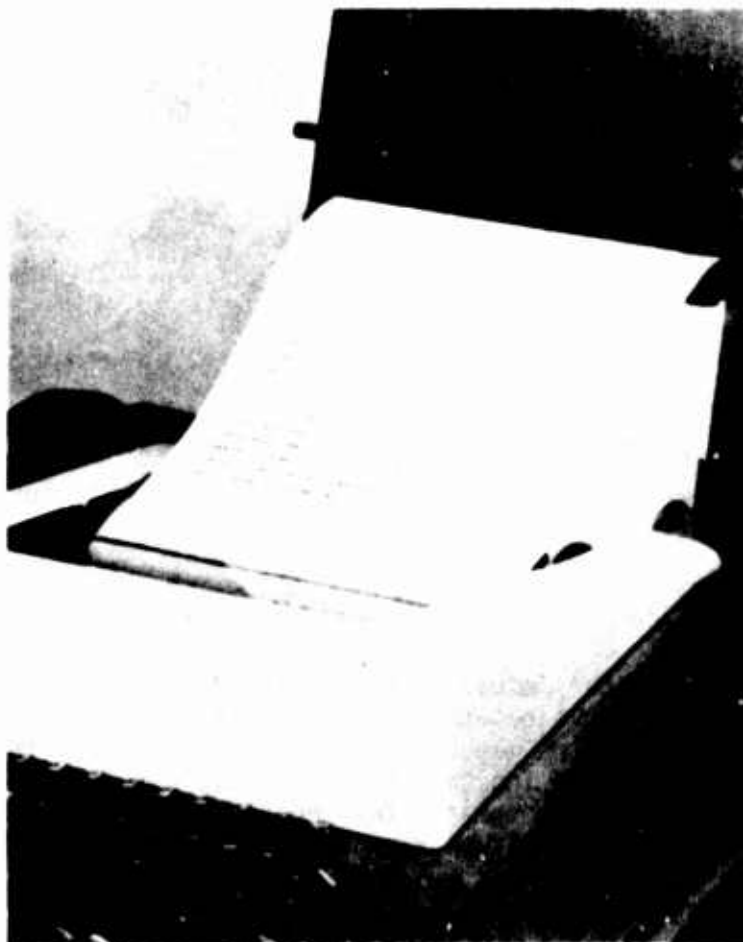


Fig. 15. Section of teletype output.

Figure 16 is a photograph of the computer, teletype terminal, and Test Result Reader. This system configuration, without the AutoAnalyzer, can be used to analyze filter paper with previously deposited agglutination patterns, which provides a diagnostic check on the system as well as verifying previously determined manual results.

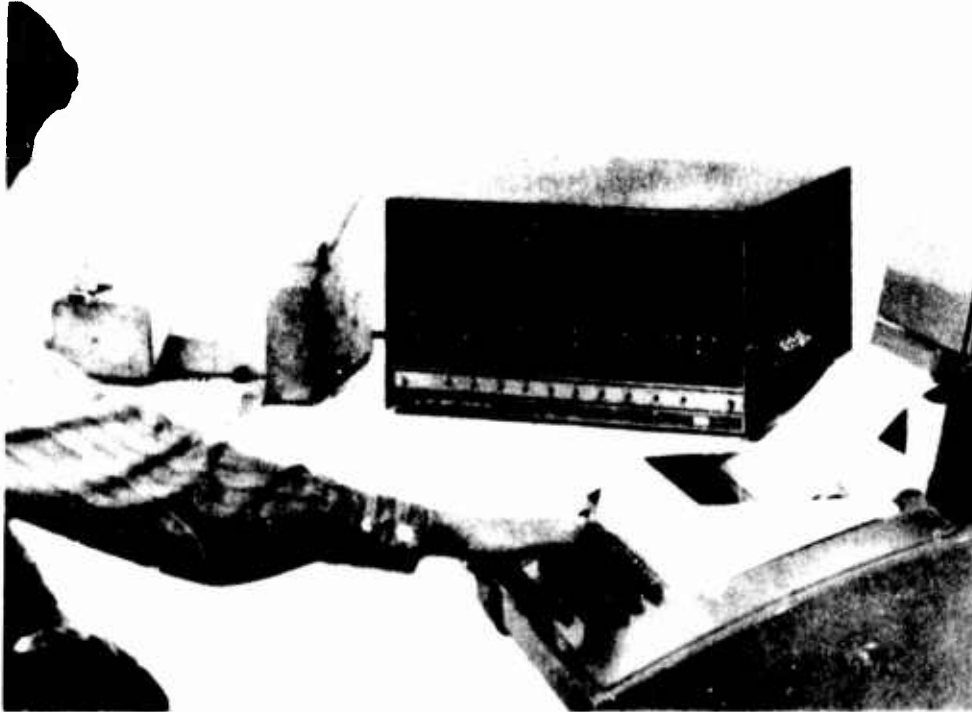


Fig. 16. Computer, teletype terminal, and Test Result Reader.

DISCUSSION

The US Army Medical Research and Development program has provided the basic design and development of a sample identification system that will eliminate error-prone manual activities associated with the identification of samples. The implementation of this sample identification design, coupled with the automatic test result analysis, provides the basis for a donor/recruit information file, providing blood typing histories, roster sheets, laboratory summary reports, and rare source files. The major features of the Automated Military Blood Typing System are:

- a. Test Result Readers which will automatically read up to 15 channels of AutoAnalyzer output directly from the filter paper record currently in use.
- b. Sample identification units which will automatically encode Vacutainers with machine and human readable numbers and which will read these numbers automatically during AutoAnalyzer testing.
- c. A means of automatically supplying blood type tags for labeling whole blood bags.

d. Automatic generation of roster sheets for use in the preparation of recruit tags.

e. Complete status and daily summary reporting of all blood typing operations.

f. Donor/recruit information file providing rapid access to blood analysis histories.

Donor/Recruit File

The system capability will be expanded to produce automatic generation of roster sheets for recruit identification tags. The removal of repeated manual transcriptions of recruit blood type information will significantly reduce errors in this process.

The donor/recruit file is implemented using additional peripheral equipment under control of the system minicomputer. The system utilizes an entry terminal for input, a magnetic disc for storage, and a report printer for output. Thus, the teletype is always available for operator communication related to system operation, and most of the computer's core memory is devoted to program management and blood analysis.

Donor Information Entry Terminal. An electronic data entry terminal will be implemented to enter donor/recruit information. All vital information, such as name, social security number, and religious preference, are entered into the terminal, as well as blood number. The correlation between social security number and blood number is stored in the data processing system and provides permanent donor identification. This terminal will be located in the blood collection area. The information can, therefore, be entered into the system while the medical history cards are being filled out.

The system can also be easily expanded at a later date to accept data from mark-sense donor cards. These cards would be automatically read by a card reader, and the information would be transferred into the data processing system. Another possible future expansion would include charge card-like identification plates with social security number imprinted, again providing automatic data transcription into the system. A photograph of the donor information entry terminal is shown in Figure 17.

Magnetic Disc Storage. Mass information storage, coupled with fast access times, will be accomplished by the use of a Magnetic Disc Console. This desk top unit, approximately 10" x 19" x 28", can store up to 1.25 million words of information (16 bits per word) on a single 15" diameter disc.



Fig. 17. Donor information entry terminal.

The exact format of the donor/recruit information storage will be determined by working in close coordination with Fort Knox Blood Bank Center personnel. The format will be kept flexible, however, so that changes can be made at any time. Table 1, below, shows a typical format for information organization. Assuming approximately 1.1 million words

TABLE 1
Typical Format for Information Organization

<u>Item</u>	<u>Format</u>	<u>Disc Words</u> (16 Bits Each)
Blood Number	6 digits	2 words
Donor/Inductee I.D. Number (Social Security)	10 digits	2 words
Donor/Inductee Name	20 alpha characters	10 words
Religious Preference	2 alpha characters	1 word
Blood Type	3 bits group 2 bits Rh 11 bits future expansion (serology, etc.)	1 word
Manual Test Results, Status Indicators	to be determined	1 word
		17 words/donor

available for data storage, over 60,000 donor/recruits could be accommodated by this format. At the present blood processing rate at Fort Knox, each disc would maintain two years of donor/recruits on file. Several discs will be used to comprise the file at the Fort Knox Blood Bank Center, US Army Medical Research Laboratory.

Storage for longer terms will be accomplished by storing discs in logical order. Data on the discs can be automatically rearranged for more effective retrieval, such as the transferring of rare blood sources to a special disc. Also, obsolete data, such as discharged donors, can be removed from storage at periodic intervals. A future expansion of this system might include transferring data from disc to IBM compatible tape at regular intervals. This tape could then be processed at other military computer centers and integrated into military files as necessary. A photograph of the Magnetic Disc Console is shown in Figure 18.

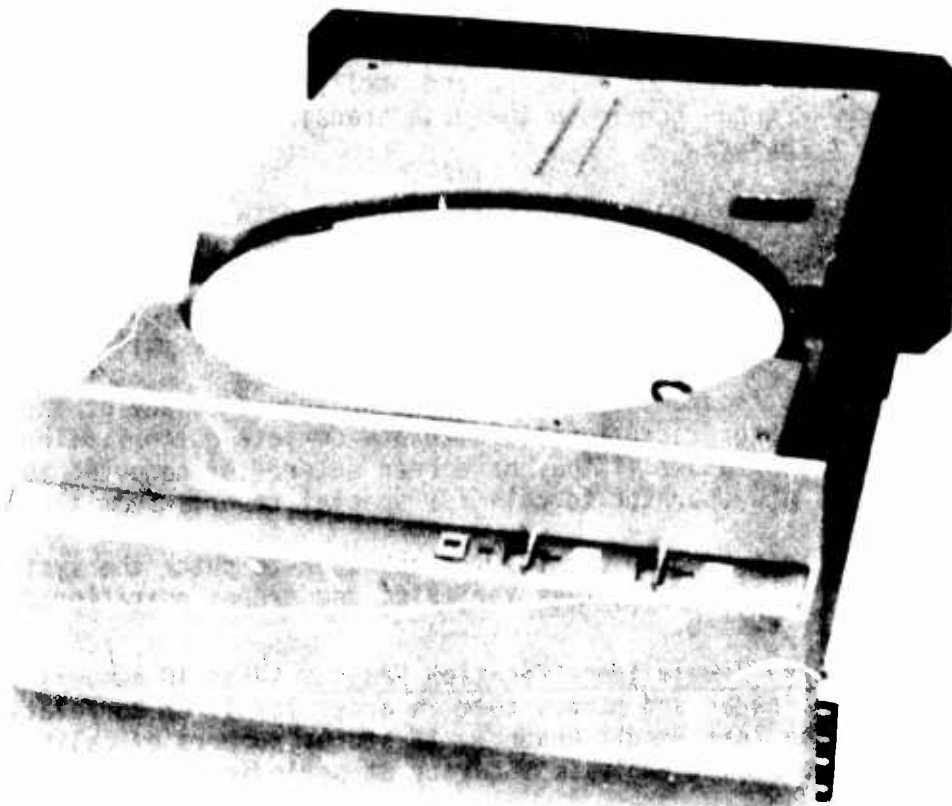


Fig. 18. Magnetic Disc Console for mass information storage.

Report Printer. A printer will be used to generate daily summary reports, roster sheets for recruit identification tag preparation, and reports as requested from the data processing system. It will be a desk top, 75 line per minute device.

Data Processing Hardware

A Master Control Program regulates the entire operation of the hardware and software system. It is comprised of two parts: The Executive (Control) Program, and the Input/Output (I/O) Servicing Program.

a. The Executive Program constantly tests the peripheral equipment to see if it requires servicing, and provides services to the other programs in the system. For example, if a program wants to output data to the printer, it requests access to the printer by signalling the Executive Program. The Executive Program takes the data, outputs it to the printer, and signals the other program that the task has been completed. This allows the original program to continue processing without "worrying" about outputting and inputting data, thus using the computer program unit more efficiently.

b. The Input/Output Program actually communicates with the outside world. It manipulates the hardware and notifies the Executive Program when the hardware has completed the data transfer and/or is ready to accept a data transfer.

A Report Generation Program sorts all the data from the donor/recruit file and controls the outputting of all reports.

The Data Entry Program allows the operator to enter data (e.g., name, ID number) into the computer for updating the donor/recruit file.

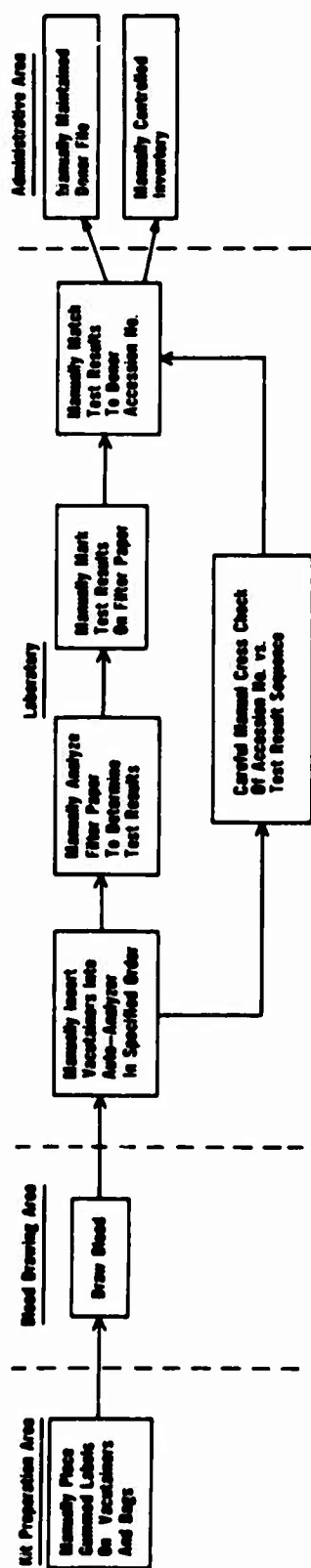
The Inquiry Program allows the operator access to any of the data entries in the donor/recruit file, with appropriate authorization code, and to change the values if they have been entered or computed incorrectly. It allows the operator to call for special reports from the file.

The Self Test Program permits the operator to check the system using a predefined set of values, thus verifying the proper operation of the entire computer system.

The Positive Sample Identification Program takes ID numbers read by the Vacutainer Reader and stores them in order for later correlation and print-out by the Test Result Reader. It stores numbers of calibrating standards for checking analysis accuracy at periodic intervals.

Blood Bank System Operation

The blood banking process will be considerably streamlined by the Automated Military Blood Typing System. Figure 19 compares the present process to the automated donor/recruit typing process.



Present Donor Typing System

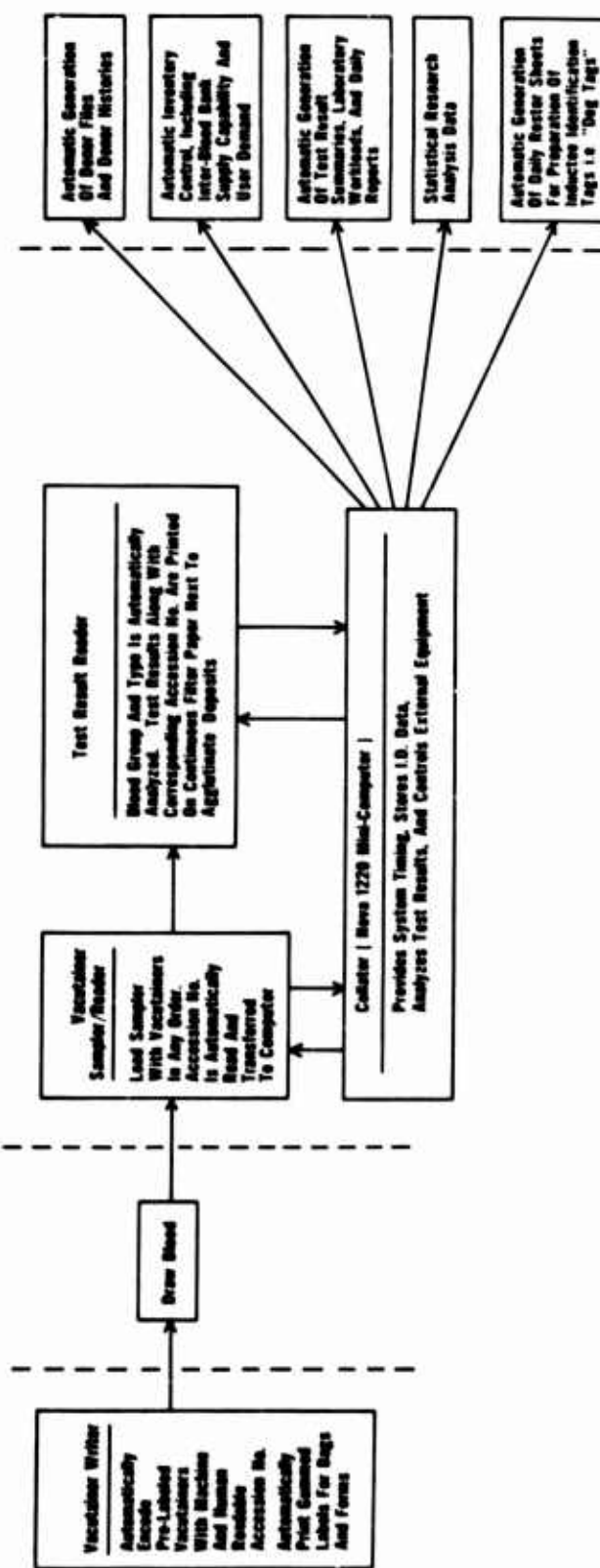


Fig. 19. Automated military blood typing system with positive sample identification.

Kit Preparation Area. In the present system, the blood collection kit is prepared by sticking gummed labels on each item involved in the blood collection process. In the automated system, a Vacutainer with a preaffixed label is inserted into the Vacutainer Writer. The blood number, a six digit accession number, is thermally written on the label in both machine and human readable code. The number is read by the machine to verify its validity. Then, a series of pressure-sensitive labels is printed by the Vacutainer Writer. These labels are placed on the blood bag, donor card, and sample collection containers used for manual testing (e.g., hepatitis). A blood collection kit containing a machine readable pilot Vacutainer has now been prepared.

Blood Drawing Area. In the present system, each donor/recruit is matched with a blood number in the blood drawing area. The social security number is marked on the donor card, which is the only record of the correlation between recruit and blood sample. In the automated system, donor/recruit information is typed into the system via the donor information entry terminal. The terminal communicates with the operator, verifying the information. The donor-blood sample correlation is now stored on the magnetic disc at the time the blood is drawn, providing positive sample identification throughout the blood drawing process.

Laboratory. Referring to Figure 19 in the present system, Vacutainers must be loaded in specified order into the Technicon turntable. The blood chemistry reactions are then initiated, resulting in continuous filter paper with a series of agglutination deposit patterns. Next, a technician manually analyzes these patterns to determine the blood types, and marks the results next to the deposits on the filter paper. This task is often performed by two people to attempt to minimize human error. Finally, a careful check between samples and results verifies the blood number of each sample. The blood numbers are marked or stamped by hand onto the filter paper. In the automated system, the Vacutainers are loaded into the Vacutainer Reader in any order. This instrument automatically reads the blood numbers from the Vacutainer labels and aspirates the samples into the system. The blood type results are automatically analyzed and printed with corresponding blood number on the filter paper. No manual analysis or additional sample identification is necessary.

Administrative Area. In the present system, blood numbers are manually correlated with recruit identification numbers, and this information is used to prepare a roster. This list is used to label the units of blood and prepare recruit identification tags. In the automated system, the roster is generated automatically by the Report Printer, along with other pertinent reports and summaries. In addition, donor/recruit histories may be maintained indefinitely. Also, statistical research on results of blood screening can be performed.

SUMMARY

In general, the automated system eliminates errors resulting from tedious manual transcription. It saves time and effort by permitting blood to be analyzed in unspecified order due to the prenumbered machine readable samples. It saves time, labor, and errors by performing automatic analysis of blood group and type. In addition, the system eliminates paper work with its donor/recruit file. Perhaps most important, it stores vital donor/recruit information which can be recalled in a moment's notice at any time.

In summary, the Automated Military Blood Typing System, which will be field tested in 1973 at the Fort Knox Blood Bank Center, has the following features:

- a. Positive sample identification.
- b. Readout/print-out of test results.
- c. Automatic supply of tags for labeling plastic blood bags.
- d. Donor records which are automatically maintained.
- e. Availability of inter-blood bank inventory data base.
- f. Statistical research may be performed on a continual basis.
- g. Donor records and rare blood donor files are created and maintained.
- h. Daily reports and summaries of testing are automatically generated.

Finally, there is generation of error-free rosters for preparation of military identification tags.

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SECTION III

PICTORIAL REVIEW

of

US ARMY MEDICAL RESEARCH LABORATORY

MILITARY BLOOD PROGRAM

PERSONNEL

and

ASSOCIATES

COMMANDERS

US ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY

1961 - 1973



SVEN A. BACH, COL, MC
Sep 61 - Aug 64



ROBERT J. HOAGLAND, COL, MC
Sep 64 - Jan 67



RALPH H. FORRESTER, COL, MC
Jun 67 - Aug 69



NICHOLAS F. CONTE, COL, MC
Sep 69 - Jun 72



FRANK R. CAMP, JR., COL, MSC
Jul 72 - Present

EXECUTIVE OFFICERS
US ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
1965 - 1973



T. A. CARILIA, COL, MSC
Jun 60 - May 66



H. H. CRAIG, COL, MSC
Jul 66 - Aug 68



R. C. LOYD, COL, MSC
May 69 - Aug 70



J. B. GIPSON, LTC, MSC
Dec 70 - Nov 73



J. R. BREWER, LTC, MSC
Nov 73 - Present

US ARMY
BLOOD TRANSFUSION RESEARCH MILITARY STAFF 1965-1973
FORT KNOX, KENTUCKY



HOAGLAND, R. J.
1964-67



CAMP, F. B., JR.
1965-73



SHIELDS, C. E.
1965-70 1971-73



FORRESTER, R. H.
1967-69



CONTE, M. F.
1969-72



DAUBER, L. G.
1966-68



REED, L. J.
1966-68



BLUM, H. F.
1965-68



KAPLAN, H. S.
1967-69



LITWIN, S. D.
1967-69



DAWSON, R. B., JR.
1968-71



LOFAS, H.
1968-71



BIMENDORF, M. L.
1968-71



BELL, C. E., JR.
1968-71



MALLIN, W. S.
1971-73



ROTH, G. J.
1971-73



POSKITT, T. R.
1971-73



BOBROW, J. C.
1971-73

RESEARCH SCIENTISTS, (BIOCHEMISTRY—IMMUNOLOGY)



W. F. KOCHOLATY, Ph.D., Director,
Biochemistry



F. DeVENUTO, Ph.D., Principal Investigator,
Biochemistry



A. J. LUZZIO, Ph.D., Principal Investigator,
Immunology



G. L. MOORE, Ph.D., Principal Investigator,
Biochemistry

Contributions, teaching, and guidance in biochemistry and immunology for all areas of research, operations, and training poured forth from these dedicated scientists (1965-1973).

SICKLE CELL RESEARCH GROUP 1969-1973



MAKIO MURAYAMA, Ph.D.
Research Biochemist,
National Institutes of Health



ROBERT H. NADELMANN, M.D.
Associate Pathologist, Stedman Memorial
Hospital; Associate Professor of Physiology,
Wayne State University School of Medicine;
US Army Medical Research Laboratory, Ft. Knox, Ky.



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Professor of Physiology
Wayne State University School of Medicine



BRUCE NICHOLS, B.S.
Chemist, Stedman Memorial Hospital



MARION I. BARRANGER, Ph.D.
Professor of Physiology,
Wayne State University School of Medicine



FRANK R. CAMP JR., COL, MC
CD/Doctor, US Army Medical Research Laboratory;
Director, The Blood Bank Center, Ft. Knox, Ky.;
Adjunct Assistant Professor of Biology,
Bowling Green State University, Ohio



JAMES H. LYMAN, M.D.
Associate Professor of Pathology,
Wayne State University School of Medicine



NICHOLAS F. COVE, COL, MC, (M.D.)
Chief Medical Consultant,
Office of The Surgeon General,
Department of the Army
Washington, D.C.



WALTER H. SOTHERS, Ph.D.
William D. Tuttle Professor of Hematology;
Professor and Chairman, Dept of Physiology,
Wayne State University School of Medicine



T. H. EVANS, M.D.
Professor and Chairman, Dept of Gynecology
and Obstetrics, Wayne State University
School of Medicine



JAMES M. GITTEN, Ph.D.
Professor of Biochemistry,
Wayne State University School of Medicine

CONSULTANTS, ADVISORS AND FRIENDS



P. LEVINE



J. M. BLUMBERG



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A. S. WISNER



S. F. BLUMBERG



S. H. SWISHER



F. H. ALLEN, JR.



C. M. ZIMMERMAN



S. B. LEVIN



E. E. ROBINSON



J. M. BIRNBAUM



S. M. HALBANDIAN



F. B. BLAIR



J. B. KIRBY



ELISE H. HAYES



E. E. RACE



RUTH SANDER



A. CHANUTHI



T. J. GREENWALT



P. J. SCHMIDT



E. E. JENNINGS



A. E. MOURANT



C. F. VORDE BRUGGE



F. LIONETTI



J. L. HANSEN



A. G. GELS



D. B. RENDICK



T. C. JEFFERS



I. DAVIDSON



M. MURAYAMA



J. R. BOYE



Miss Laura Reynolds

In the capacities of Clerical Assistant, Administrative Assistant, Secretary, and Security Control Officer, Miss Reynolds has served each Commander from 1950 to the present in an efficient and dedicated manner. In 1962 she was commended by The Surgeon General, DA. During her early years of Federal Service she received the Decoration and Award for Meritorious Civilian Service.



Mrs. Rachel G. Bertram, Secretary to the Director, The Blood Bank Center, worked through the early planning stages of the translation series in blood group immunology and the many monographs and special studies. This required massive amounts of correspondence, research, grit, and patience. She is known to scientists throughout the world for her conscientious efforts and high standards of editorial expertise.



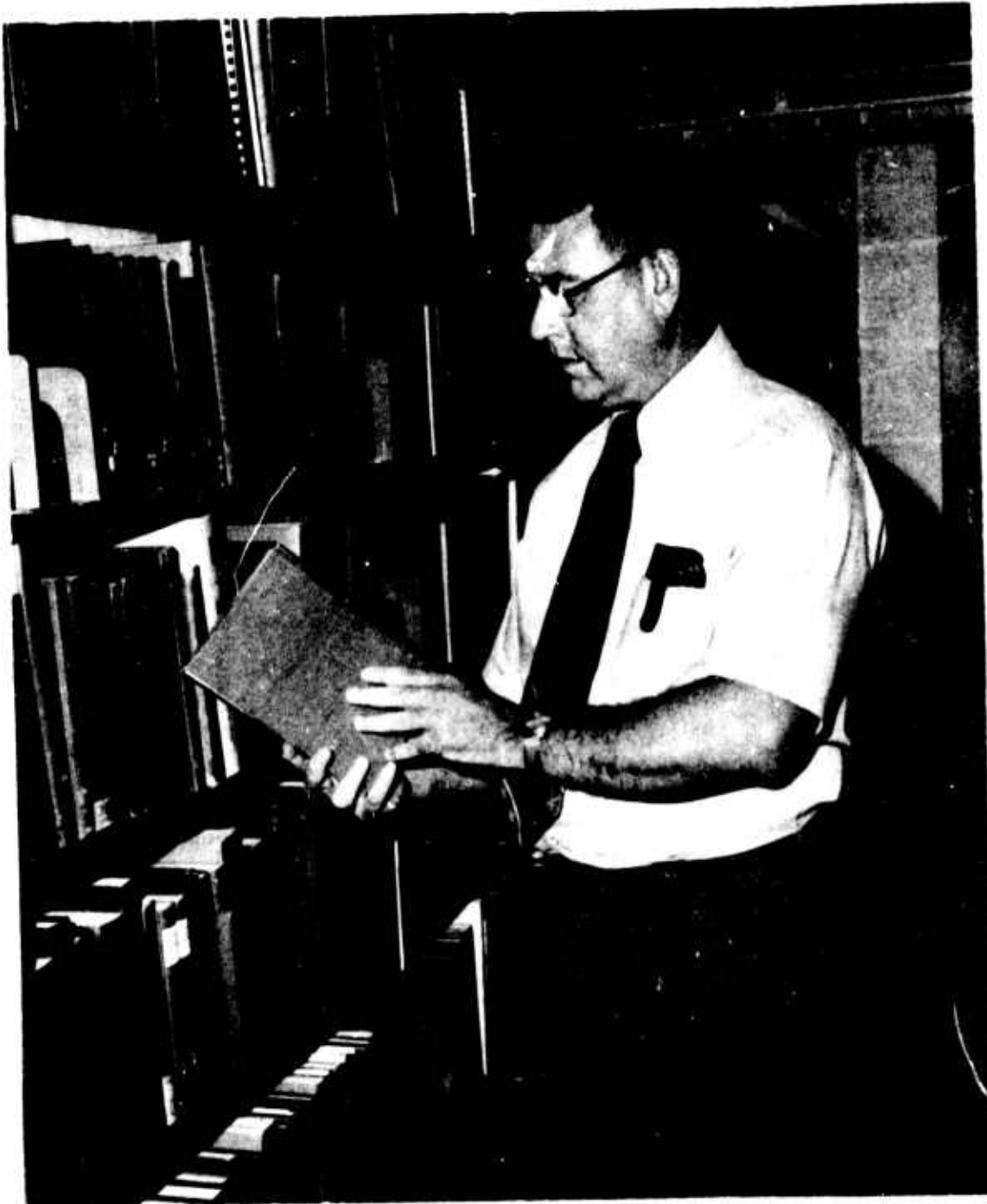


Mrs. Bertram and Mrs. Cynthia S. Carter have worked together in preparing the many publications from The Blood Bank Center. The long hours of research and preparation have been arduous and exciting. Their reward is contained in the letters received from scientists throughout the world commending their scholarly efforts.



Captain Edward Malewski, MSC
Adjutant
1972-Present

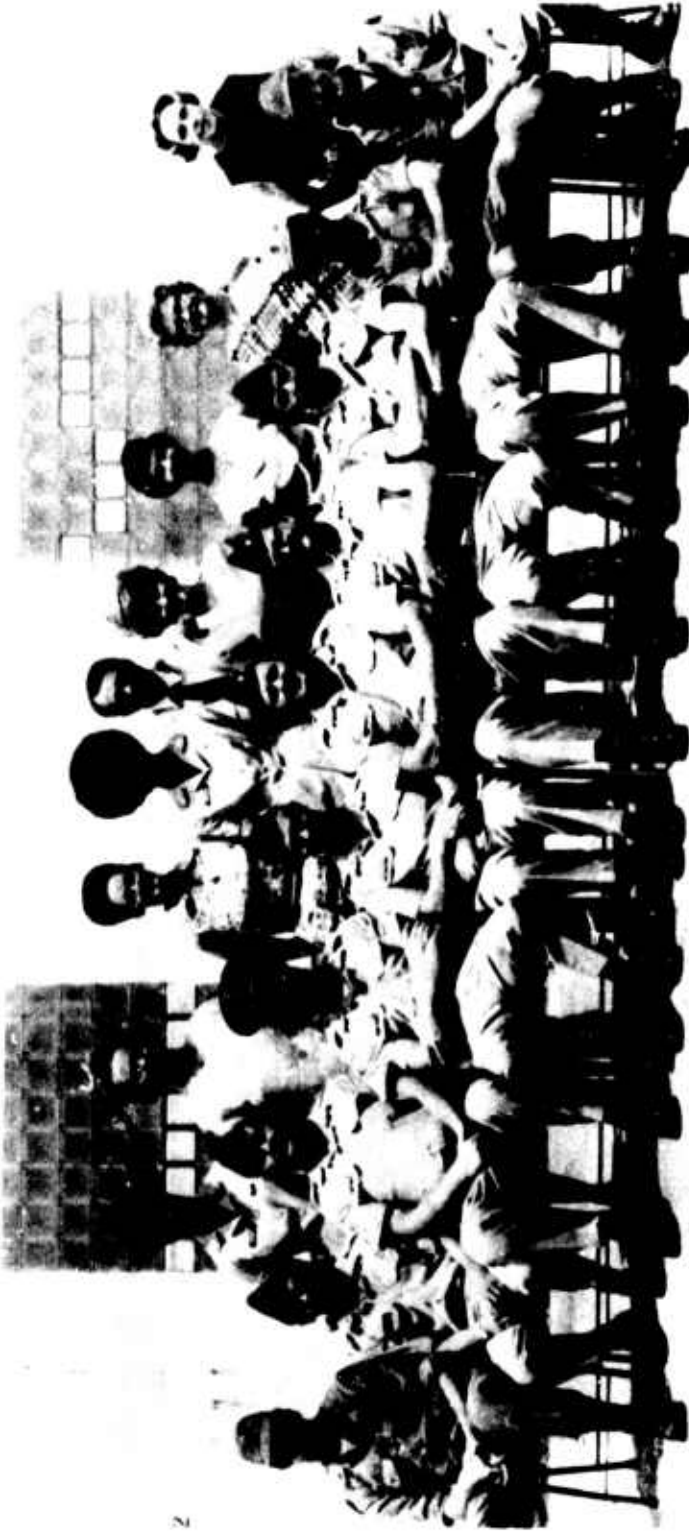
An outstanding officer who contributed substantially to the smooth transition phases between the USAMRL transfer to the Western Military Institute of Research and the phase-in of a significant expansion of The Blood Bank Center.



Henry L. Ward
Librarian
1972-Present

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HEADQUARTERS JUNE 1972

Second Row:

Loretta A. Ramsey, Thelma Singleton, Martha Davis, Lillie M. Whitten, Richard A. Wheeler, M. Ann Haraway, Cynthia Carter, Julia Judd, Dorothy Stiglitz

First Row:

Allan Lazzareschi, Lawrence O. Dean, Arl W. Thompson, James M. Hogan, Nicholas F. Conte, Joe B. Gipson, Lyle W. Warner, Raymond Kirk, Angel W. Maldonado, Gale E. Buys



BLOOD BANK CENTER MAY 1972

Fifth Row

Dwight D. Stemen, Vance L. Russell, Cosby M. Cobb, David A. Rouse, Terrance W. Donahue

Fourth Row:

Doralee M. Guthrie, Florence G. Whiteside, Norvel F. Meyer, Jr., Myron D. Nicholson, William H. Craig, John P. Gibson, Orton A. Beesley, Edward Prescott, Thomas H. Zweber, John A. Perry, Robert L. Woodland

Third Row:

Vivian L. Widman, Linda L. Benson, Myrtle E. Oldham, Christa A. Harrison, Willa M. Banks, Virginia B. Sharp, Wilson E. Johnson, Bernard T. McCutchen, John A. Nechworth, Robert T. Lawn, Jr.

Second Row:

Margaret E. McPeak, Mary Lynn Franks, Lillian W. Necessary, Ima Gene Shirley, Nancy L. Hisle, Rachel G. Bertram, Shirley C. Lingo, Debbie J. McManus, Deborah H. Hall, Margaret A. Love, M. Gail Smith

First Row:

Roy L. Miller, Kenneth I. Tobias, Virgil R. Coley, Frank R. Camp, Jr., John D. Arnoldin, John A. Naples, Antonio G. Camuze, Jr., Robert G. DeBonville, Loran R. McKinley, Jr., James H. Young



BLOOD RESEARCH DIVISION JUNE 1972

THIRD ROW:

Larry M. O'Maley, James A. Eaton, Thomas A. Rudai, Robert T. McNally, Bruce H. Blake, Stephen B. Moore, Mark L. Failla, Joseph F. Bratton, Dennis T. Reuss, William E. Kline, Raymond Grant, Angelo I. Zegna, John M. Pulley, Robert W. Kraus, Joseph L. Zapp

SECOND ROW:

Paul F. Cook, James Williams, M. Ann Wredman, M. Edith Ledford, Betty C. Allen, Gladys T. King, Beverly J. Lunsdis, Wilfredo R. Manalo, James C. Donofrio, Thomas A. Billings

FIRST ROW:

H. Philip Fortwengler, Gerald L. Moore, Gerald J. Roth, William S. Mallin, Walter F. Kocholaty, Charles E. Shields, Frank DeVenuto, Anthony J. Luzzio, Thomas R. Poskitt, Stanley C. Roberts



PATHOLOGY DIVISION JUNE 1972

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Barbara J. Grafton, Kay F. Higbee, Dorothy P. Gaston

FIRST ROW:

Issac O. VanMeter, Francis R. Johnson, Merriel R. Gardner, Jesse H. Crain, John T. Ervin, Mark G. Burns, Tom O. Moore, Joseph E. Corbett



EXPERIMENTAL PSYCHOLOGY DIVISION MARCH 1972

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John M. Scamehorn, Kevin E. DeVries, Bruce A. Bell, Delbert C. Wiley, Charles K. Bardick, Daniel A. Collins, Robert L. Thompson, Dennis E. Sheidler, Walter W. Beam, John D. Peterson, William S. Kemmerer, Robert J. Salvo, James I. Morrison, Thomas G. Smith, Eugene M. Surma, Victor J. Pribyl, E. Booker McClaskey, Otto J. Dilly, Carl E. Gaffney

Middle row l-r:

Valerie N. Rees, Johanna W. Mitchell, Mary W. Bickett, Mary A. Miller, Carol J. Padgett

Front row l-r:

John K. Schielderup, James N. Cronholm, James D. Mosko, Isaac Behar, Lee S. Caldwell, George S. Harker, Gregory W. ...
Peter A. O'Mara, George A. Luz, Frederick N. Dyer, James H. Patterson



RESEARCH SUPPORT DIVISION JUNE 1972

Third Row:

James E. Smith, Foster B. Finn, David J. Lenz, William J. Todd, Charles E. Williams, Roy E. Caruthers, Willis F. Barber, John Szekeres, Timothy Butcher, Philip E. Corbit, William J. Payne, Maxie M. Nichols, Edward E. Fulkerson

Second Row:

Birch Stevenson, Jerry Atcher, Jess Wheatley, Eugene E. Tucker, C. J. Potson, Kenneth M. Stankard, Glenn Lee, Walter W. Smith, George W. Weeks

First Row:

Francis P. Rice, Lyle W. Warner, Ernest M. McClubb, Joseph R. Nall, Joe P. Gipson, Thomas L. Watts, Joseph M. Bickett, Louise Snyder, Rose A. Lundergan



Colonel Marcel E. Conrad, MC (M.D.)

In many of our projects (operations, training, and research), Marc showed us how to get back on the road, if we strayed.

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Dr. W. B. Redmond
Assistant Professor of
Microbiology
Emory University
and
Chief, Tuberculosis Res. Lab.
Veterans Admin. Hospital
Atlanta, Georgia



Dr. W. B. Baker
Professor Emeritus of Biology
Emory University
and
Director, Emory University Museum
Atlanta, Georgia

An individual is most fortunate when his teachers and mentors remain as consultants throughout life.



Dorothy N. Sage
Director, Blood Bank
Veterans Administration Hospital
Atlanta, Georgia



LTC Walter J. Black, USA (Ret)

LTC Black provided the initial blood donor supply in 1966 for Vietnam and sustained this effort throughout his tour. This insured a highly successful blood program at Fort Knox for Vietnam requirements.



Edward T. Nolan

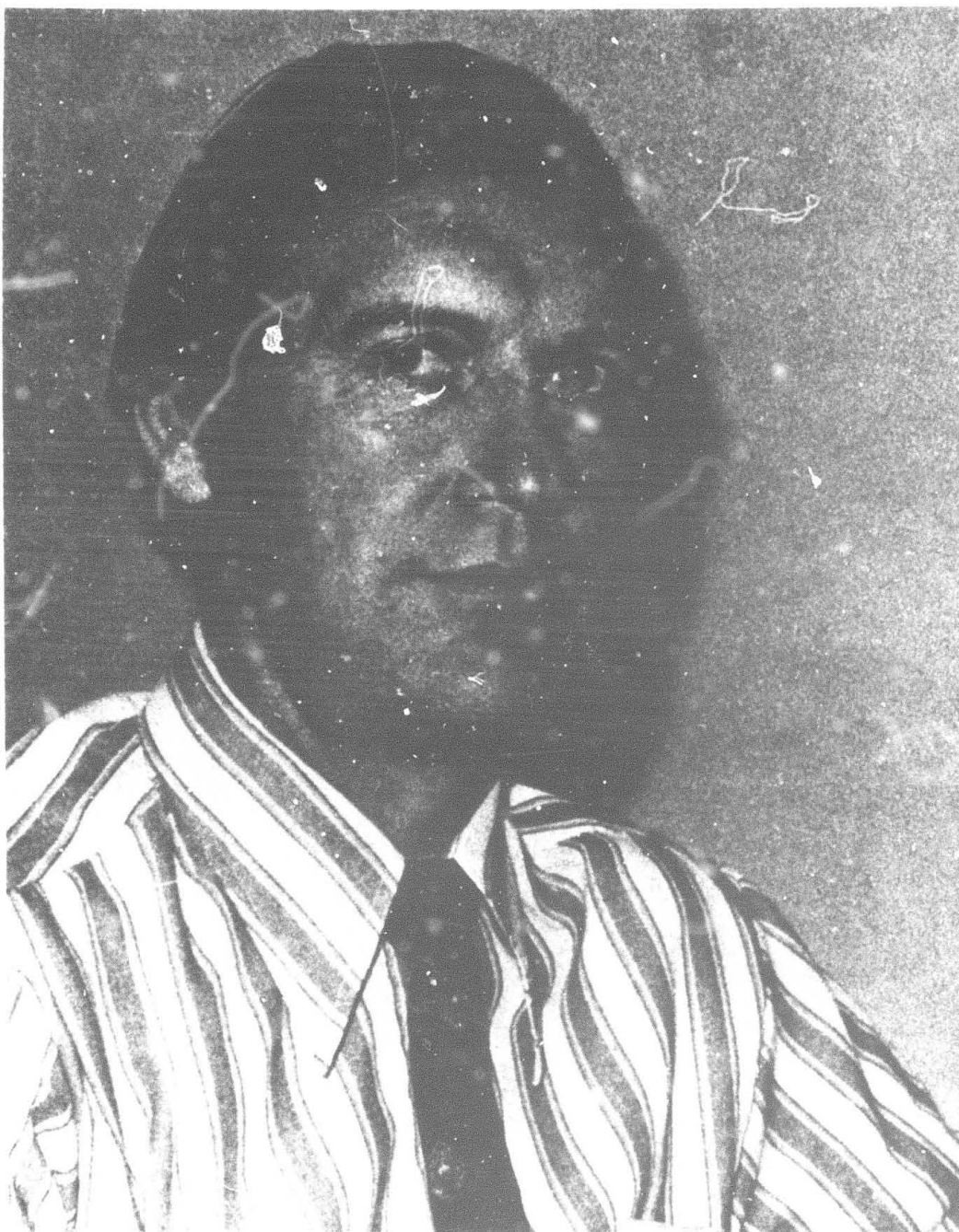
Mr. Nolan served as an Administrative Assistant in the Blood Transfusion Research Division and participated in numerous decisions during its early period of growth.



LTC George H. Seeger, USA (Ret)

LTC Seeger contributed to the success of the blood research, training, and operational program at Fort Knox by his expertise in supply, personnel, operations, and management.

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Douglas N. Huestis, M.D.

Dr. Huestis graciously advised us on matters pertaining to the training of the Blood Bank Fellows.



John G. Gibson, II, M.D.

Dr. Gibson has provided continued support of military blood banking and has made substantial contributions to the Blood Bank Fellowship Training Program and to blood preservation research.



Sam T. Gibson, M.D.

Dr. Gibson was continuously available for consultation concerning blood group reagent testing for the Armed Services.



Workshop on Quality Control of Blood Group Reagents for staff inspectors of the Defense Personnel Support Center.



COL Donald L. Howie, MC (M.D.)

Colonel Howie, while serving as Deputy Commander, US Army Medical Research and Development Command, assisted effectively in the activation of blood research, operations, and training at the US Army Medical Research Laboratory, Fort Knox, in 1965.



COL Paul A. LaVault, MSC

Colonel LaVault, while serving as Comptroller, US Army Medical Research and Development Command, assisted effectively in the activation of blood research, operations, and training at the US Army Medical Research Laboratory, Fort Knox, in 1965.